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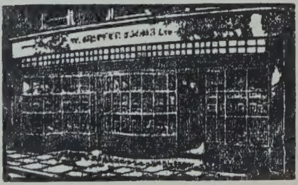

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Correlations between Growth and Flowering in *Chenopodium amaranticolor*

II. Leaf and Stem Growth

BY

RODERICK G. THOMAS

Plant Physiology Unit, D.S.I.R., Palmerston North, New Zealand

With nine Figures in the Text

ABSTRACT

Plants of *Chenopodium amaranticolor* grown in long photoperiods for 26 days were exposed to 0, 2, 6, or continuous short days (SD) and the resultant changes in leaf and stem growth on return to long days recorded. Growth of the leaves, the main stem, and the axillary branches was initially strongly stimulated by short days. Continued growth of leaves was inhibited by the development of flowers to anthesis; strong inhibition was observed after 6 and continuous SDs, but not in the 2-SD or control groups, neither of which reached anthesis during the investigation.

In the groups which flowered strongly, the intensity of inhibition was sufficient to mask completely the effect of the initial stimulation on final leaf and stem size. In the 2-SD group, however, lack of inhibition allowed full expression of the effects of stimulation on the final leaf and stem dimensions.

The system of stimulators and inhibitors controlling leaf growth probably also brings about the changes in leaf shape associated with the onset of flowering.

The results are discussed in relation to previous findings and it is suggested that similar stimulations of growth correlated with the induction of flowering might occur widely in both short- and long-day plants.

INTRODUCTION

IN many species, including *Kalanchoë blossfeldiana* (Harder, 1948), *Chenopodium amaranticolor* (Lona, 1949*b*), and *Cannabis sativa* (Thomas, 1957), photoperiod is known to influence concomitantly leaf size, leaf shape, and flowering. Whether these factors are affected independently by daylength is unknown in most cases. In the short-day plant *Sedum kamtschaticum*, the influence of daylength on leaf shape and succulence appears to be separate from its effect on flowering; in certain circumstances it has been shown that short days will influence leaf shape without inducing flowering (Meyer, 1947). In many other species, however, there is no evidence of two such separate daylength effects.

Changes in leaf growth, which become noticeable at about the stage when flower buds are nearing anthesis, are very widespread in both short- and long-day plants. Such consistent close relationships suggest that the physiological state which brings about the formation of flower primordia also affects leaf growth.

[Annals of Botany, N.S. Vol. 25, No. 99, 1961.]

In the past, investigations aimed at elucidating the relationship between flowering and leaf growth have generally, as in all examples cited above, involved measurement of mature leaves only. Results obtained from these studies have been able to show little more than the manner in which the development of flower buds towards anthesis is accompanied by an inhibition of leaf growth which leads to a considerable reduction in final leaf size (e.g. Leopold *et al.*, 1959).

The final size and form of a leaf, however, is but the summation of all phases of its growth from the time of its inception up to maturity. During that time the factors influencing these phases can be both stimulatory and inhibitory, and whether or not such situations occur can only be determined by following the growth of successive leaves right from initiation to maturity. If a combination of promoting and inhibiting phases does occur and is also closely correlated with the initiation and development of flower primordia, as the present study shows to be the case, a knowledge of these phases can assist our understanding of the processes leading to the induction of flowering.

In the present investigation an attempt has been made to avoid the interactions of the several factors which can influence leaf growth during the early stages of plant development. When plants are grown from germination in inductive photoperiods any effects on leaf growth of processes leading to a reproductive state are unavoidably superimposed on the results of these other developmental trends accompanying the loss of juvenility, e.g. changes in the rate of leaf initiation, apex diameter, and the ratio of mature to immature tissues. To overcome this difficulty, seedlings were grown in non-inductive conditions for almost 4 weeks, by the end of which time their rate of leaf initiation was steady and little further change in leaf size and shape was occurring from node to node, i.e. they were past the juvenile stage. In this way leaf growth has been related to various degrees of induction of flowering and a strong initial promotion of growth by inductive conditions has been revealed. Less detailed observations have also shown the existence of a comparable situation with regard to the growth of stems.

MATERIAL AND METHODS

The short-day plant *Chenopodium amaranticolor* was chosen for the present investigation because the initiation of flower primordia on plants otherwise grown in long days can be induced by exposure to a single long dark period (Lona, 1949*a*), and changes in leaf shape appear to be related to the onset of flowering (Lona, 1949*b*).

The experimental procedure has been described fully in the preceding paper (Thomas, 1961). Plants were grown from germination in 16-hour photoperiods (LD) until they reached an 'adjusted' age of 26 days and they were then divided into four groups to receive (*a*) continuing LDs, (*b*) continuous 8-hour photoperiods (SD), (*c*) 2 SDs followed by a return to LD, and (*d*) 6 SDs followed by LDs.

Records of the growth of leaf primordia were obtained by dissection of plants at intervals during the first 3 weeks after the start of treatments as described in the previous paper. Growth of larger leaves was followed on 8 plants per group which remained undissected through the experiment. On the latter plants, measurements of the dimensions of all visible leaves were made at approximately weekly intervals from the onset of the various photoperiodic treatments and internode lengths were measured 34 and 63 days after the start of SDs.

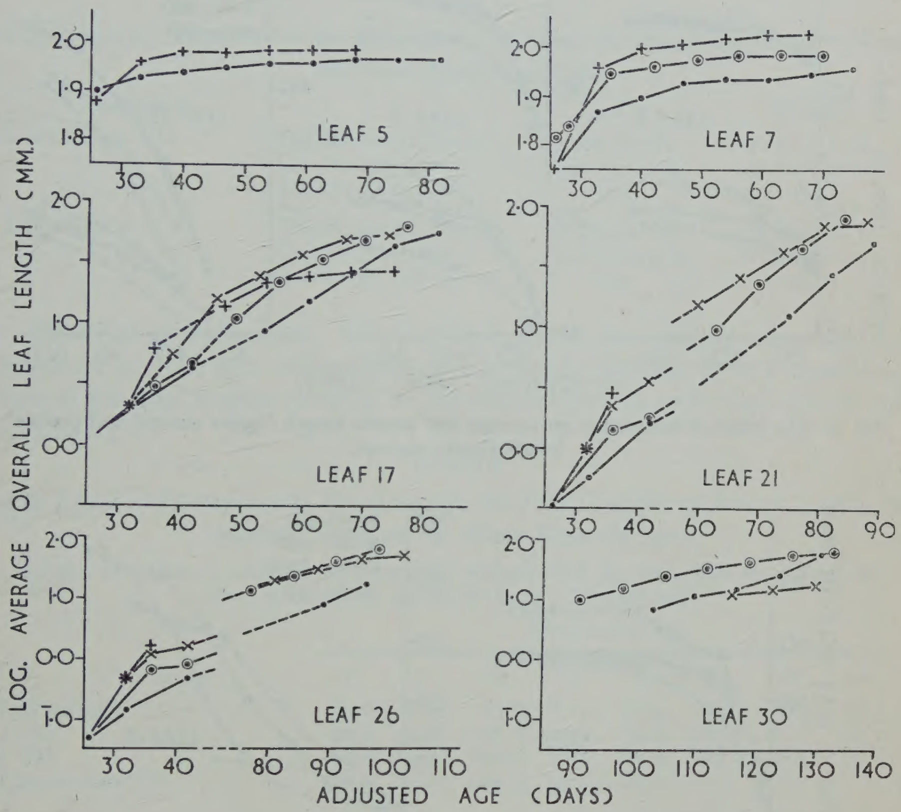

FIG. 1. The effect of various photoperiodic treatments on leaf growth (average length of lamina plus petiole) at selected nodes from 5 to 30. Early stages of growth at nodes 17 to 26 were determined by dissections; later stages at all nodes were measured on whole plants. At nodes 17 to 26, measurements made in these two ways are linked by broken lines. Clarity was maintained for nodes 5 and 7 by omitting measurements of the 2- and/or 6-SD groups. Nodes are numbered acropetally. ●——● = LD control, ⊙——⊙ = 2 SD, **x**——**x** = 6 SD, +——+ = continuous SD.

RESULTS

Leaf Growth on the Main Stem

Stimulation of leaf growth by short days. The initial influence of inductive conditions on the growth of the petiole and lamina of selected leaves from nodes 5 to 30 is shown in Fig. 1. A pronounced stimulation of leaf elongation at each node was brought about by the onset of short-day conditions and at most nodes this stimulation was apparent within 2 to 6 days of the start of inductive treatment. Stimulation was greatest in the continuous-SD and 6-SD groups, but a clear effect was also noticeable on those plants exposed to only 2 SDs.

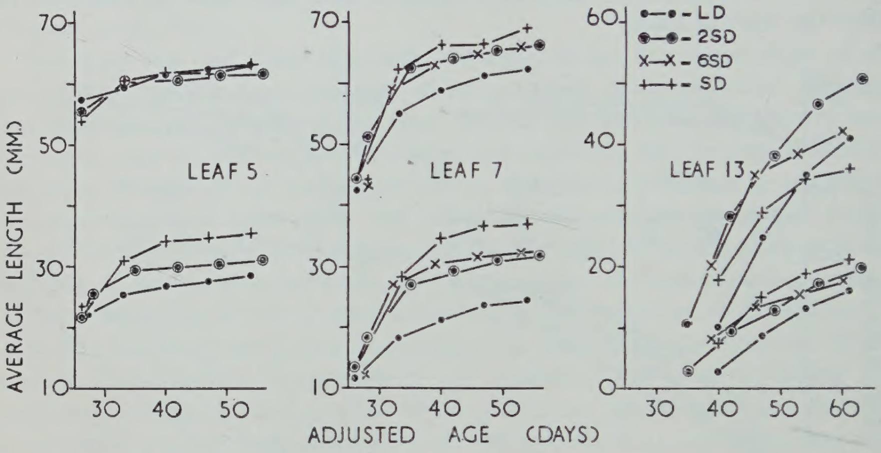

FIG. 2. The effect of treatments on average leaf lamina length (upper curves) and petiole length (lower curves).

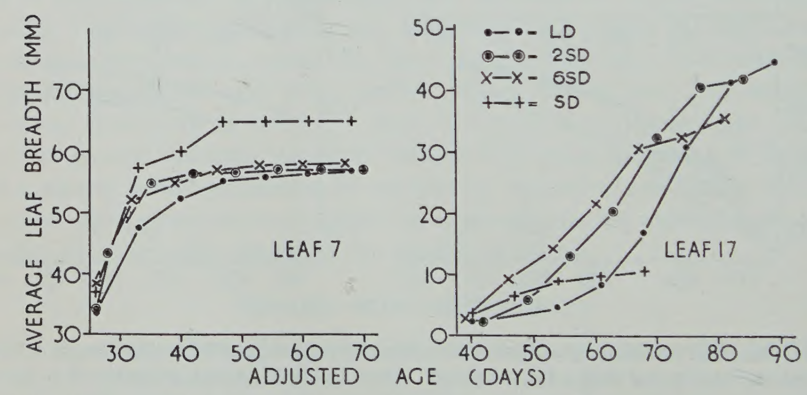

FIG. 3. The effect of treatments on leaf breadth.

It is of interest that the leaf at node 5, which had almost attained its final length before SD treatment started, was still able to respond to such a stimulus. At this stage lamina growth had nearly ceased and only petiole elongation was affected (see Fig. 2). At higher nodes, elongation of both petioles and laminae was stimulated by SDs and it is apparent that such a stimulus was not selectively confined in its action to a single region of the leaves. Leaf breadth was affected in an exactly comparable manner (see Fig. 3).

How much growth stimulation was brought about by cell expansion and how much by cell division is not certain, but measurements of the epidermal cells of the petioles at nodes 5 and 6 in the LD and continuous-SD groups indicate that the main influence was on cell division (Table 1). These data suggest that in *Chenopodium*, as in *Lupinus* and *Helianthus* (Sunderland, 1960), cell division can contribute to leaf expansion up to a very late stage of its development.

TABLE 1

The Effect of Induction on the Relationship between the Final Lengths of Petioles and their Epidermal Cells

Treatment		Leaf 5	Leaf 6
Continuous SD	Petiole length (mm.)	39·00±0·81	41·25±1·96
	Cell length (mm.)	0·070±0·003	0·072±0·002
	Estimated cell no.	557	573
LD control	Petiole length (mm.)	29·00±1·33	30·00±0·47
	Cell length (mm.)	0·066±0·002	0·066±0·002
	Estimated cell no.	439	454

Inhibition of leaf growth. Although leaf growth was initially stimulated by exposure to SDs, such a stimulation is not readily apparent from a consideration of the final lengths attained by successive leaves in each treatment group (Table 2).

TABLE 2

The Effect of Photoperiodic Treatment on the Final Lengths of Foliage Leaves at Selected Nodes on the Main Stem (in mm.)

Leaves at nodes 26 and 30 were not fully elongated at the time these measurements were made (when the plants were 130 days old)

Treatment	Node numbers							
	5	7	10	13	17	21	26	30
LD control	93·6	91·5	93·5	72·4	73·7	93·1	80.5	52·3
2 SD	92·8	99·6	108·5	91·0	73·6	103·5	87·7	62·6
6 SD	90·9	101·3	100·0	76·4	65·9	86·0	72·7	18·4
Continuous SD	102·4	110·6	101·7	59·1	27·0	—	—	—

The stimulatory effect of SDs is only noticeable in this table at node 7. The final overall lengths of these leaves were least in the LD controls and greatest in the continuous-SD group, those of the 2-SD and 6-SD groups being intermediate.

At node 10 and above, treatment with SDs eventually had a definitely inhibitory influence on the growth of leaves. The final size attained by the leaves of the continuous-SD group decreased rapidly above this node and at node 21 leaves were too small to measure accurately without magnification. At nodes 10 and 13 in the other groups, however, the stimulatory effect of SDs was still apparent in the final leaf size, but whereas the initial stimulation increased with increasing exposure to SDs (see Fig. 1), final leaf size was considerably greater in the 2-SD than in the 6-SD group.

Beyond this point the final size of the leaves of the 6-SD group fell below that of the LD group and the difference between the leaves of the 2-SD group and the LD controls became slight.

Thus, although SD treatment had a pronounced stimulatory effect on leaf growth initially, its final influence was inhibitory. This inhibition was greatest with exposure to continuous SDs, less after 6 SDs, and not apparent during the course of the experiment following exposure to 2 SDs, but whereas stimulation of leaf growth first occurred prior to the development of differentiated flower primordia, the onset of inhibition was very closely related to the beginning of anthesis (as measured by the first emergence of anthers from the enclosing perianths).

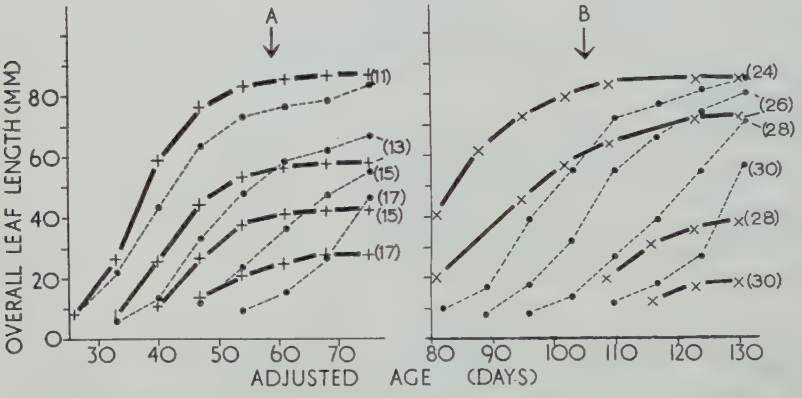

FIG. 4. The relationship between leaf growth and anthesis. ●- - - -● = LD control, +——+ = continuous SDs, x——x = 6 SDs. Arrows at A and B indicate the average time of anthesis in the continuous- and 6-SD groups respectively. Node numbers are bracketed.

The closeness of this relationship in the continuous-SD and 6-SD groups is shown in Fig. 4: a rapid cessation of leaf growth throughout the plants clearly accompanied the later stages of flower development. In the 2-SD group, which did not reach anthesis before the end of the experiment, there was no significant inhibition of leaf growth relative to the LD controls (see Table 2). Continued observations of this group beyond the experimental period, however, showed that anthesis eventually occurred and that a concomitant inhibition of leaf growth was clearly associated with this stage of development.

When strong inhibition of the growth of a leaf occurs during its primordial stage its further development into a foliage leaf is prevented. As a result, bract formation occurs and observations in the present investigation indicate the dependence of this upon the stage of reproductive development attained by neighbouring tissues.

At the start of SD treatments an average of approximately 26 leaf primordia had been initiated on the main stem of each plant. In the group of plants exposed to only 6 SDs the lowest node at which bract formation occurred ranged from 27 to 32. On the other hand, with more vigorous growth and development of flower primordia in the continuous-SD group, strong inhibition of leaf growth resulting in bract formation occurred at all nodes above the nineteenth. Thus it appears that this inhibition acting at nodes 19 to 26 in continuous SDs must have occurred after exposure to the first 6 short photoperiods. By the end of the sixth SD, however, double ridges had already been initiated in all the groups receiving SD treatment and it therefore seems unlikely that factors leading to the formation of double-ridges could have exerted an inhibitory influence on leaf growth.

On the other hand, although the differences in the degree of stimulation of the formation of double ridges between the variously induced groups were slight, differences between the rates of flower development were very great (Thomas, 1961). The rate of flower development thus seems to be far more closely linked with the time of bract formation, and the results in Fig. 1 show that the growth of leaf 17 in continuous SDs was not inhibited in any measurable way until 30 days after the start of treatment, by which time flower primordia were fully developed and well on the way to anthesis.

Leaf shape. Changes in leaf size brought about by the various inductive treatments were accompanied by changes in relative length and breadth. Observations of the changes which occurred during leaf development (Fig. 5) showed the final differences in leaf shape to be the result of at least three apparently independent effects:

(*a*) At the lower nodes, viz. node 17 and below, SD treatment led to an increased relative elongation of a leaf during its final stages of growth. At node 17 this was hardly noticeable after 2 or 6 SDs, but there was a strong effect of continuous SDs. The same effect was apparent at node 21 in the 6-SD group, but it was not detectable at higher nodes.

This final decrease in the relative breadth of leaves immediately preceding the cessation of growth seems to have been closely related to a late stage of flower development for it was absent in the 2-SD group and most apparent after continuous-SD treatment. Such an effect probably resulted from the action of the leaf growth inhibitor shown to be associated with flower bud development immediately preceding anthesis. At the late stages of leaf growth, petiole elongation continued for some time after lamina expansion had ceased (see Fig. 2). It is thus quite likely that the initial effect of the inhibitor would have been the complete suppression of lamina expansion, leading temporarily to an increase in relative overall leaf length.

(*b*) At higher nodes (node 21 and over), at which leaf primordia were absent, or less than 1 mm. long at the start of SD treatment, the initial effect of all SD treatments was the stimulation of elongation relative to breadth. This effect was equally strong in 6- and continuous-SD groups but weaker in the 2-SD group. The excessive relative elongation was gradually lost during ontogeny, but leaves remained relatively shorter on the LD controls.

As treatment with 2 SDs seems to have had at least a slight effect on the elongation of leaf primordia, and such treatment was sufficient to bring about the general stimulation of leaf growth described earlier, it is quite probable that the increase in relative length of leaf primordia following exposure to SDs was the result of this general stimulation. That the effect of such stimulation on these young leaves was primarily an enhancement of elongation could be explained by the observation that the ratio of leaf breadth

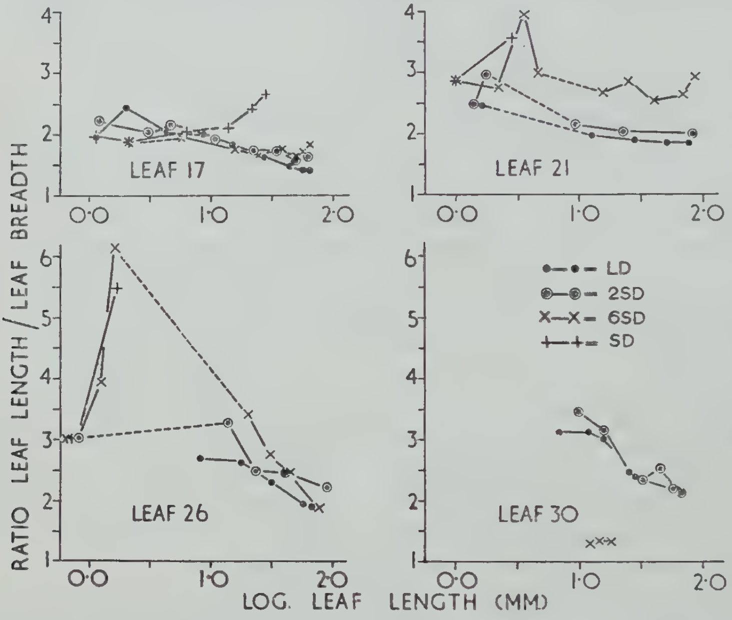

FIG. 5. The effect of treatments on the change in ratio of leaf length to breadth during growth of selected leaves from nodes 17 to 30. Measurements of young leaves at nodes 17 to 26 were made by dissections; older leaves were measured on whole plants (at nodes 17 to 30). Measurements made in these two ways are linked by broken lines.

relative to length increased during leaf ontogeny (Fig. 5). It follows from this that during the early stage of leaf development cell division contributes more to the length than the breadth of a primordium. A stimulation of cell division at such a stage would then lead to an increased length/breadth ratio.

(*c*) At node 30, and several nodes above and below it, at which leaf primordia were not initiated until after the start of SDs, exposure to 6 SDs led to the development of leaves which showed a great increase in relative breadth during their final growth stages. Reference to Fig. 6 shows this to have been the result of decreasing elongation concurrent with continued growth in breadth.

This phenomenon is not readily explicable on the basis of a simple stimulation/inhibition hypothesis. A similar type of leaf abnormality associated with the transition from a reproductive to a vegetative condition brought about by a sudden change from short to long photoperiods has been observed in *Cannabis sativa* (Schaffner, 1926; Thomas, 1957). The sudden switch of strongly induced plants into conditions favouring vegetative development probably disturbs the balance of morphogenetic regulators and thereby leads to abnormal leaf development. The basis for the production of these deformities has yet to be elucidated.

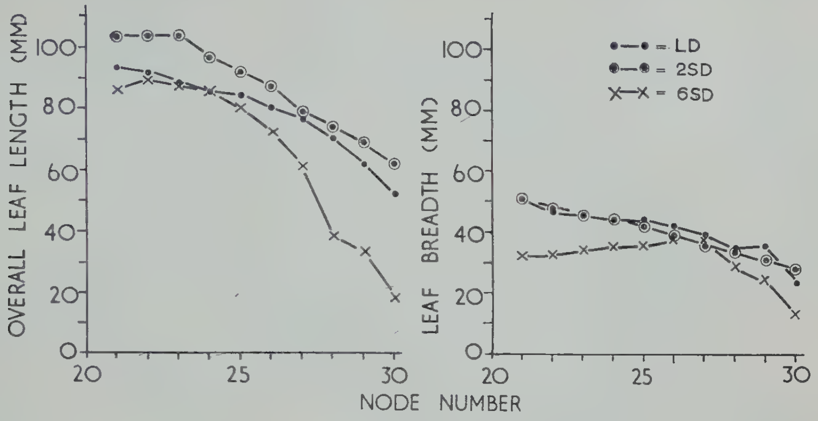

FIG. 6. The effect of treatments on final leaf length (left) and breadth (right) at nodes 21 to 30.

The changes in leaf shape accompanying the transition to flowering, apart from the abnormal development of the leaves near node 30, can thus be explained in terms of the simple stimulator/inhibitor system which regulates leaf growth during this stage of development. There is no need to postulate a specific 'leaf shape' factor.

The Growth of Leaves on Axillary Branches

The effect of inductive treatment on the growth of the leaves on lateral branches and the main stem was comparable. Leaf growth was stimulated during the early stages of floral initiation, but as flower development advanced an inhibition set in. This situation is shown clearly by measurements made 63 days after the start of inductive treatments (Fig. 7*a*, *b*).

The initial stimulation of leaf growth increased with increasing SD treatment, but in the continuous-SD group the onset of the later inhibitory period was very rapid with the result that leaf growth on the axillary shoots above node 11 on the main stem was less than in the LD controls. This inhibition occurred much later in the 6-SD group and, at the time when the measurements were made, leaves in this group were all larger than in the LD controls. Comparison with the 2-SD group, however, shows growth of the leaves on axillary branches above node 20 to have been inhibited in response to 6-SD treatment.

That the rates of leaf appearance on axillary branches and the main stem were influenced in the same way by different inductive treatments is shown by Fig. 7*b*, in which the average number of axillary leaves longer than 3 mm. is recorded. Although these measurements do not represent total numbers of leaves on the lateral branches, they do show that increasing duration of SD treatment led to a more rapid appearance of leaves. In the continuous-SD and 6-SD groups, inhibition of leaf growth on axillary branches led to an apparent fall in the number of leaves initiated on those above nodes 8 and 12 respectively. Leaf initiation on axillary branches did not decrease above nodes 8 and 12 in this way; the results obtained were brought about by an inhibition of leaf growth above these points.

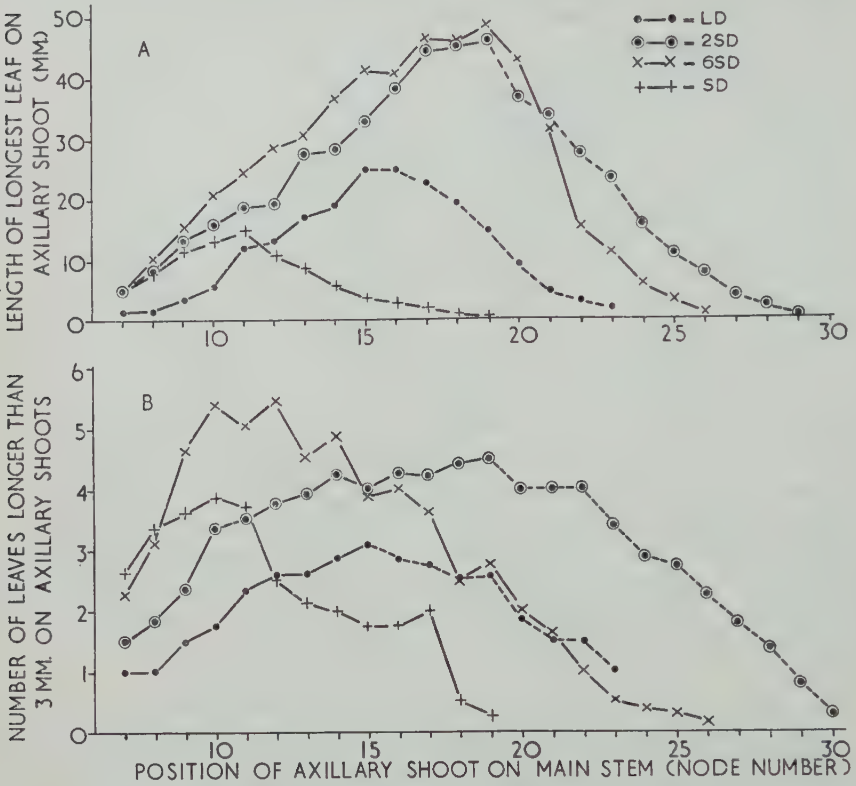

FIG. 7. The effect of treatment on the growth of axillary shoots. Measurements were made 63 days after treatments began. Measurements of axillary shoots at which growth had ceased are connected by unbroken lines. Broken lines join the points in regions which were still immature at the time of measurement.

Growth of the Main Stem

The elongation of internodes on the main axis responded to SD treatment in a way similar to that previously described for leaf growth. Fig. 8 shows the effect of various treatments on the internode lengths 34 days after the start of short photoperiods. Internode elongation was stimulated by inductive treatment and exposure to as few as 2 SDs had little less stimulating effect than continuous SDs in comparison with the LD controls. This stimulation influenced internodes which had begun to elongate before the start of inductive treatment (i.e. 2 and 3) as well as those which were still very short at the beginning of treatment (i.e. 5 and above).

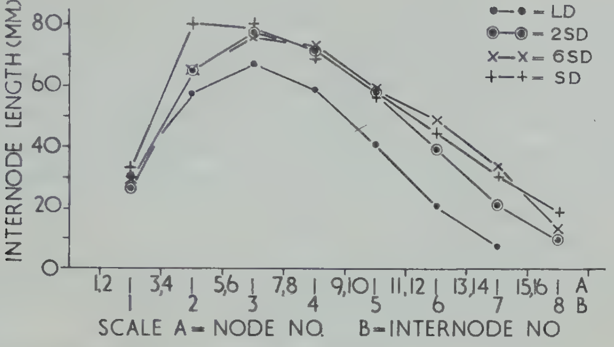

FIG. 8. The effect of various photoperiodic treatments on internode length. Measurements were made 34 days after treatments began.

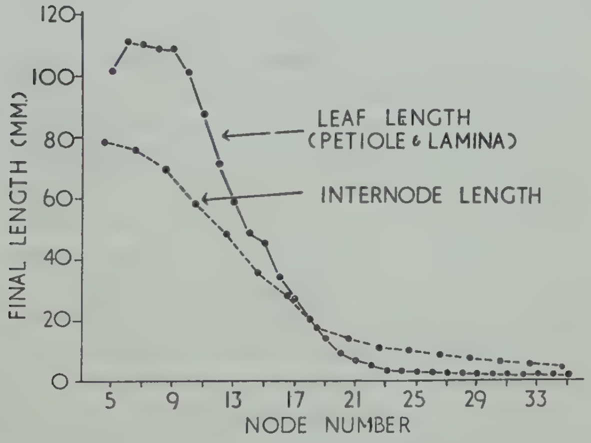

FIG. 9. The effect of exposure to continuous SDs on the final lengths of leaves and internodes.

The inhibitory influence associated with the onset of anthesis was less marked in relation to stem growth than leaf growth. Thus, in the continuous-SD group, leaf primordia did not develop beyond the bract stage at node 19 and above, so that the final length of leaves at node 23 was only 2 per cent. of the maximum attained lower down the stem, but internode elongation was proportionately much less inhibited. The final length of internode 12, immediately above node 23, was on the average 10 per cent. of the maximum internode length attained lower down the stem (Fig. 9).

It seems quite probable that stem elongation in relation to flowering is controlled by at least a third factor in addition to an initial stimulus and a later-formed inhibitor. Such a factor might be produced at the time of synapsis within the flower primordia. Wittwer (1943) showed that in *Spinacia* a clear promotion of stem elongation in male plants occurred at this stage and observations on *Cannabis* (Thomas, 1960*a*) indicate a similar relationship to occur in the latter. *Cannabis* is a caulescent plant which produces flower buds irrespective of daylength, but in which SDs are necessary for anthesis. Rapid stem elongation occurs in this genus concomitant with the onset of the rapid growth of flower buds. It thus might well be that in *Chenopodium* the inhibitor which has such a strong influence on leaf growth affects internode elongation in the same way, i.e. it has a widespread inhibitory influence within the plant, but that its effect on internode elongation is masked to a certain extent by the formation of a further growth promoter produced at a still later stage of flower development.

As was the case with leaf growth, internode elongation on axillary and main stems responded to the treatments applied in comparable ways.

DISCUSSION

Observations of apical organogenesis, described in part I, showed the rates of initiation of leaf and axillary bud primordia at the apex in LDs to be stimulated by exposure of the plants to SDs. Measurements of leaf growth, stem elongation, and growth of axillary shoots, reported in the present paper, show that a comparable stimulation of the growth of these tissues was induced by SD treatment. It is not improbable that all these stimulations are manifestations of a single developmental change induced by short photoperiods.

The relationship between leaf growth and flowering. The relationship between apical growth and the transition to flowering has been discussed in the previous paper. That the stimulation of leaf growth is also closely related to the onset of flowering is suggested by the following observations:

(i) The intensity of flowering and the degree of stimulation of leaf growth are influenced in the same way by the duration of SD treatment; in both cases the effect of continuous SD $>$ 6 SD $>$ 2 SD $>$ LD.

(ii) Exposure to as few as 2 SDs results in an immediate stimulation of leaf growth and a concomitant transition to flowering. The effect of such a brief stimulus on leaf growth is long-lasting and is still apparent several weeks after LD treatment has been resumed. It thus seems that once a plant is induced to flower, leaf growth is promoted regardless of daylength.

(iii) An experiment comparing the effects of SDs with and without interrupted dark periods (Thomas, 1960*b*) has shown that interruption of the dark period with a light-break prevented both flowering and the stimulation of leaf growth.

How widespread is the occurrence of this growth stimulation in relation to flowering remains to be seen, but at least two sets of previous results on re-examination appear to agree with these here reported for *Chenopodium*. Schwabe (1956) published the results of some experiments with *Xanthium* showing the effect of three different photoperiods on leaf area. Some of his results are summarized in Table 3 from which it is readily apparent that a stimulation/inhibition hypothesis provides a completely adequate explanation. A comparison of the slowly flowering 14-hour group with the continuous-light controls shows growth of the former to have been considerably greater, and therefore more rapid (the data given being the leaf sizes after a given time and not the sizes of the fully expanded leaves), above leaf 6. In the rapidly flowering 8-hour group, however, leaf growth was stimulated from the fifth leaf upwards but inhibited, presumably as a result of strong flowering, from leaf 8 upwards. Thus the basic stimulation of leaf growth in the 8-hour group was probably greater than in the 14-hour group, but the superimposition of a stronger inhibition in the former group obscured this promotion above node 7.

TABLE 3

The Effect of Photoperiod on Leaf Growth in Xanthium (*after Schwabe, 1956*)

Hours light per day	Days to flowering	Leaf area per leaf number					
		5	6	7	8	9	10
8	16·5	14·0	18·1	19·0	17·7	12·0	8·9
14	43·0	12·5	16·0	18·0	22·6	25·8	26·4
24	—	12·4	16·2	15·3	13·8	8·0	1·4

This situation is exactly comparable with that recorded in the present investigation of *Chenopodium* where leaf growth was clearly less stimulated in the 2-SD than the 6-SD or continuous-SD groups, but consideration of final leaf size only would give the impression that stimulation was greater in the former group.

Bünning and Konder (1954), in studying the relationship between leaf growth and flowering, concluded that the leaves of plants grown in inductive conditions were smaller and grew more slowly than those of plants grown in non-inductive conditions. In most cases the plants grown did not flower during the course of their investigations and their results are not really relevant to the present discussion, but in the case of the short-day plant, *Perilla*, flower initiation occurred as a result of SD treatment. Re-examination of the graphs showing leaf growth in LDs and SDs in this plant shows clearly that the growth of the lower leaves was more rapid in SDs than LDs even though the final leaf size was considerably greater in LDs (possibly as a result of inhibition?).

Little evidence is available at present with regard to the relationship between leaf growth and floral initiation in long-day plants, but recent studies of the influence of LDs on plants of *Spinacia* and *Trifolium repens* transferred from SDs (unpublished results) have shown that inductive conditions lead to a stimulation of leaf growth in these plants also. It seems quite likely that such a relationship is very widespread.

In the present investigation, the initial stimulus to leaf growth was followed by an even stronger inhibition which was closely related to the onset of anthesis. Similar inhibitions of leaf growth accompanying flowering have been noted in many other plants regardless of their daylength requirements (e.g.

Borrill, 1959; Leopold *et al.*, 1959) and evidence suggesting a close relationship is thus strong. It is quite possible that the level of growth inhibitors within developing flower buds increases when they reach a certain stage just prior to anthesis. The finding that male spinach inflorescences contain rather large amounts of growth inhibitors (Bulard and Leopold, 1957) supports this suggestion.

The relationship between stem growth and flowering. Evidence for the occurrence of a stimulation of stem elongation concomitant with the start of inductive conditions in other short-day plants besides *Chenopodium* is less readily available, but re-examination of some results on Biloxi soybean (Parker and Borthwick, 1939) shows clearly that stem elongation was increased by exposure of plants, otherwise grown in LDs, to 7 SDs. Over the following 4 weeks the elongation in plants which received SDs averaged 63·2 cm. while that of the LD controls was 49·2 cm. When plants were maintained in SDs for a longer period, however, this promotion of growth was completely overshadowed by a very strong inhibition which led to the stimulation passing unnoticed.

The bolting of rosette plants, in which sudden and rapid stem elongation is closely associated with the onset of the reproductive phase, should not perhaps be considered comparable with the stem elongation in caulescent plants; bolting has been shown (Sachs *et al.*, 1959) to result from the stimulation of cell division in the sub-apical region of the stem, whereas in caulescent plants divisions in this region probably occur under all natural conditions. In addition, bolting can be induced without a concomitant induction of flowering (Wellensiek, 1960).

It was suggested earlier (Thomas, 1961) that the onset of inductive conditions might bring about the release of an inhibition within the plant apex and that the resultant stimulation in turn might lead to the initiation of double ridges. The results cited in the present paper show that this stimulation is not always confined to the apical tissues, but that it can also be manifest throughout all the growing regions of the plant.

ACKNOWLEDGEMENTS

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Spore Discharge in *Deightoniella torulosa* (Syd.) Ellis

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With five Figures in the Text

ABSTRACT

Violent spore discharge in the Hyphomycete *Deightoniella torulosa* is described. On drying, a negative pressure develops in the solution inside the swollen head of the conidiophore. This causes the thin wall at the top of the conidiophore to cave inwards and to be brought into a state of tension. The sudden appearance of a gas phase in the conidiophore head releases this tension, and the wall rapidly returns to its original rounded shape. The conidium, which is attached to the top of the conidiophore, is thus catapulted away. This appears to be the first record of a gas phase being associated with violent spore discharge in a fungus.

INTRODUCTION

THE fungus *Deightoniella torulosa* is widely distributed throughout the tropics (C.M.I. Distribution Map No. 175) as a common parasite and saprophyte on bananas. It is responsible for a 'black-spot' disease of leaves (Ashby, 1913; Stahel, 1934; Meredith, 1961*b*), 'black-tip' disease of fruits (Ogilvie, 1928; Wardlaw, 1932; Meredith, 1961*b*, 1961*d*) and spotting ('speckle') of fruits (Meredith, 1960, 1961*a*, 1961*b*). In a saprophytic capacity the fungus occurs on decaying banana pseudostems, leaves, bracts, and flower parts in the plantation.

Several mechanisms of violent spore discharge are known among the Phycomycetes, Ascomycetes, and Basidiomycetes. However, as Webster (1952) points out, only a few Fungi Imperfecti are known to discharge spores violently, *Nigrospora sphaerica* (Webster, 1952), *Zygosporium echinosporum* (Mason, 1941), and *D. torulosa* (Meredith 1961*c*) being examples. The present paper gives a more detailed account of spore release in *D. torulosa*, and attempts to elucidate the mechanism.

MATERIALS AND METHODS

The majority of observations were made on conidia formed on banana petioles naturally colonized by *D. torulosa*. Freshly collected decayed petioles were washed thoroughly and then incubated for 24 hours in a damp chamber maintained at room temperature (75–90° F.). After this period it was found that large numbers of conidiophores were bearing mature conidia. Thin strips of tissue were peeled away from the petiole surface and examined under a low power (×200) microscope objective, using bright, transmitted light.

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Other observations were made on cultures of *D. torulosa* growing on potato-dextrose agar.

STRUCTURE OF CONIDIAL APPARATUS

Hughes (1952) proposed the genus *Deightoniella* for didymosporous and phragmosporous Dematiaceae, having conidiophores that elongate by successive proliferations, and in which the conidia are formed singly as blown-out ends at the apex of the conidiophore and at each proliferation. Wardlaw (1932) and Ellis (1957) have described the conidial apparatus in *D. torulosa*, but it is desirable to redescribe it here and to note a few additional features.

Conidiophores arise directly from the epidermis of infected banana petioles, and are often densely aggregated over relatively large areas. They are erect, simple or occasionally branched, straight or flexous, septate and acquire a brownish colour from an early stage. The apex of the conidiophore is swollen into a bulb, 12–18μ in diameter, on which a single conidium is borne. There may be as many as 8 subglobose swellings along the conidiophore, representing successive proliferations. Conidiophores are 40–170μ long and 6–10μ thick. Proliferation of the conidiophore may occur by continued growth through the old conidial scar. If the conidium is not discharged, the conidiophore may grow on from a point below and to one side of this conidium. Wardlaw (1932) notes that occasionally two branches are formed, one on each side of the first-formed conidium.

Conidia are formed by a process of budding at the tip of the conidiophore. The new cell enlarges rapidly and a transverse septum appears towards the distal end. Additional septa and pseudosepta then develop. On infected banana tissues the conidia at maturity are straight or slightly curved, obpyriform to obclavate, subhyaline to smoky olive and 2–6 septate. Measurements recorded were 30–38 × 16–25μ; Wardlaw (1932) notes that considerably larger conidia may be formed on certain culture media. At the base of the conidium there is a dark coloured disc or scar where attachment to the conidiophore head was made.

The structure of the mature conidiophore head is shown diagrammatically in Fig. 1A. Except for an area at the top of the apical swelling the conidiophore wall is relatively thick and darkened. When viewed from above it is seen that the diameter of the circular area of thin wall is slightly greater than that of the conidial scar (Fig. 1C). The inside of the conidiophore appears to be filled with a watery solution. The conidial wall comprises a thin chitinized exosporium and a thick endosporium (Fig. 1B). The thickened scar at the base projects for some distance so that when attached the conidium is delicately perched on the conidiophore head.

OBSERVATIONS ON SPORE DISCHARGE

Thin strips of petiole bearing conidia of *D. torulosa* were taken from a damp chamber and mounted on a dry glass slide. Spore discharge commenced immediately on transfer to the microscope stage, the conidia appearing as though they were flicked from their conidiophores. Within 60 seconds more than 90 per cent. of the apparently mature conidia had been discharged. The maximum recorded horizontal distance to which conidia were projected was estimated to be approximately 2 cm. Discharged conidia do not appear to adhere to a glass surface and a slight air current is sufficient to detach them.

Other pieces of petiole tissue were wetted before taking them out of the damp chamber. No spore liberation occurred for several minutes. Later, presumably as the tissues dried out, liberation started. The effect of drying on liberation was further indicated in pure cultures of *D. torulosa*. With the lid of the dish in place no liberation occurred: removing the lid was soon followed by violent discharge of conidia.

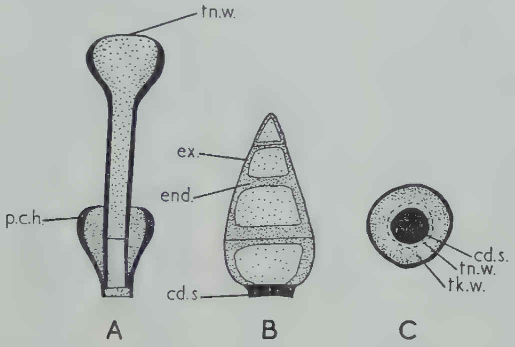

FIG. 1. A, distal end of conidiophore of *D. torulosa* showing variation in wall thickness; B, conidium of *D. torulosa*; C, conidiophore head viewed from above (all ×600). *tn.w.*, thin apical wall; *p.c.h.*, previous conidiophore head; *ex.*, exochitin; *end.*, endochitin; *cd.s.*, conidial scar; *tk.w.*, thickened wall.

Observations carried out in a dark room indicated that liberation is not dependent on light.

Attempts were made to elucidate the mechanism of spore release. Before discharge occurs the conidiophore frequently undergoes a series of movements. There is a slow bending over (Fig. 2B) followed by a rapid recovery to the original position (Fig. 2A). Just before the recovery stroke takes place a small gas bubble appears in one of the cylindrical cells of the conidiophore (Fig. 2B). This bubble enlarges rapidly and two menisci move to opposite ends of the cell, coming to rest a short distance from the septa (Fig. 2C). Similar gas bubbles appear in rapid succession in other cylindrical cells, and each time there is a rapid movement of the conidiophore. Eventually all the cells have a vacuolated appearance (Fig. 2D). These movements rarely result in liberation. Even when they do, the conidium merely falls away from the conidiophore coming to rest a few microns away. Thus some other process seems to be responsible for the violent liberation mentioned earlier.

The terminal cell of the conidiophore is usually the last to develop a gas bubble. The first reliable indication that liberation is about to occur is a partial retraction of the conidium into the conidiophore head, due to the thin area of the wall caving inwards. The forces which cause this retraction appear to be unevenly distributed since the conidium moves jerkily from side to side before resting temporarily (Fig. 3 A, B, C, D). No other visible changes occur in the conidiophore and the condition of the conidial apparatus just prior to liberation is as illustrated in Fig. 3D. A small gas bubble then suddenly appears inside the conidiophore head, usually to one side (Fig. 3D). Almost simultaneously the thinner region of the conidiophore wall springs back to its original position with the result that the conidium is shot away (Fig. 3E). The gas bubble enlarges rapidly and a meniscus is seen to move down the conidiophore head and come to rest close to the septum (Fig. 3 F, G). Examination of many conidiophores immediately after discharge nearly always revealed a rupture in the thin wall at the apex (Fig. 3 F, G). It was not possible to determine at what stage this rupture occurs. It may occur (1) just before the appearance of the gas bubble, as a result of the inward pull: or (2) after the appearance of the gas phase, due to the sudden increase in volume.

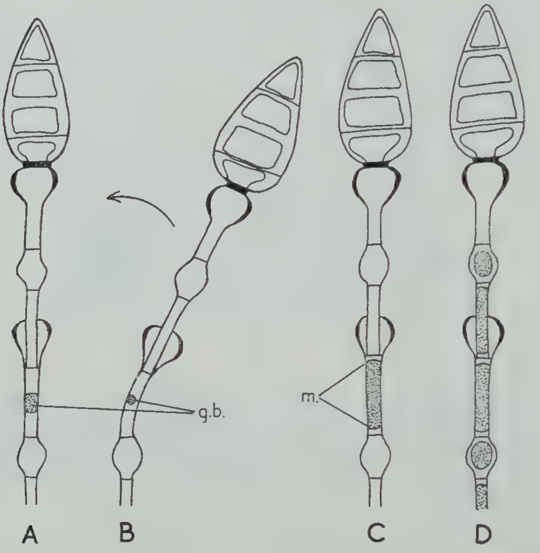

FIG. 2. A, original and final orientation of conidiophore (gas bubble not apparent at the former time); B, orientation after slow bending movement, gas bubble developed just before sudden return to A; C, D, increase in size and number of gas bubbles (all ×600). *g.b.*, gas bubbles; *m.*, meniscus.

It is only a fraction of a second between the time of first appearance of gas bubbles in the conidiophore cells and that of liberation. No further movement of the conidiophore occurs after spore release.

In Fig. 3 it is indicated that gas bubbles may also appear in the several compartments of the conidium. There is no precise order of appearance of these bubbles, sometimes those at the distal end developing before those at the base, and vice versa. Initially it was considered that the appearance of a gas phase in the conidium may have some significance in relation to the discharge process. However, the fact that gas bubbles do not always appear in the conidium before discharge tends to discredit this contention. In contrast, the appearance of a gas phase in the conidiophore head invariably accompanies discharge and this seems to be of great significance.

Caving-in of the thin wall area at the apex of the conidiophore was easily observed in newly formed conidial heads on which conidia had not yet been formed (Fig. 4 A, B). The concavity disappeared with the development of a gas bubble, the wall finally appearing convex outwards, and ruptured (Fig. 4C).

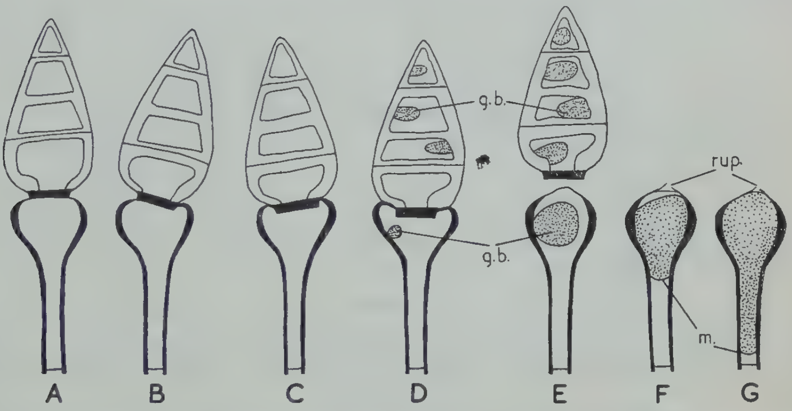

Fig. 3. A–G, appearance of conidiophore and conidium during and after the discharge process: explanation in text (all ×600). *g.b.*, gas bubble; *m.*, meniscus; *rup.*, ruptured wall.

When dry, discharged conidiophores were mounted in water, the gas bubble in each cell disappeared within 30–60 seconds and all cells became filled with solution again. A similar disappearance of bubbles was observed in discharged conidia. If the conidiophores and conidia were allowed to dry again, gas bubbles once more appeared in the manner illustrated in Figs. 2 and 3. An interesting feature was frequently noticed in the conidiophore head. The apical wall did not seem to cave in to the same extent as in undischarged conidiophores: further, the gas bubble invariably appeared at the top of the head, just below the ruptured wall area, and not to one side.

Repeated reversal of the gas phase process was brought about by alternate wetting and drying. Similar observations have been described by Ingold (1939) in the annulus cells of fern sporangia, and in ascospores of certain fungi (Ingold, 1956). He considers the gas bubble to be a vacuum saturated with water vapour. This is supported by the fact that the bubble disappears rapidly on mounting in water. Air bubbles of the same size in water are much more persistent.

Gas bubbles appear if conidiophores are mounted in water and flooded with excess concentrated glycerine. Reflooding with water results in disappearance of the bubbles.

A different sequence of events was observed in very young conidia, after transfer from a saturated to a dry atmosphere. The conidiophore underwent the characteristic side to side movements, but the conidium was not subsequently discharged. Instead the conidium became completely evaginated and drawn back into the conidiophore head (Fig. 5 A, B). During evagination the thin conidial wall was frequently ruptured.

Apparently mature conidia are not always discharged after the appearance of the gas phase. When such conidia were mounted in water and then allowed to dry out for a second time, discharge frequently took place. Perhaps the first attempt at discharge was insufficient to break the conidial attachment, and merely weakened it. In the second attempt more energy would be available for projection. If this hypothesis is correct, then it is probable that rupture of the thin apical wall occurs after liberation (as suggested in (2) above). If it occurred before final release, as in (1), it seems doubtful whether the broken wall could develop a tension sufficient to cause projection.

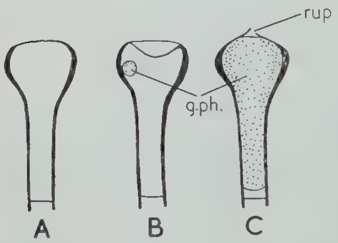

FIG. 4.

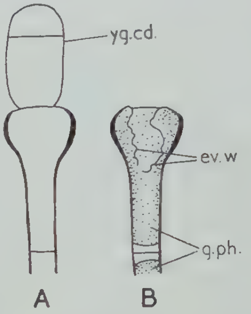

FIG. 5.

FIG. 4. A, B, development of a gas phase and distortion of the apical wall in a young conidiophore of *D. torulosa* in which a conidium has not yet developed; C, final appearance of same (all × 600).

FIG. 5. A, B, evagination of young conidium (all × 600). *ev.w.*, evaginated wall; *g.ph.*, gas phase; *rup.*, ruptured wall; *yg.cd.*, young conidium.

DISCUSSION

From a consideration of these observations on spore discharge in *D. torulosa*, the following mechanism is tentatively postulated. When a mature conidiophore, with its attached conidium, is transferred from a saturated atmosphere to a drier one, water evaporates from the interior of the terminal cell. The volume of the cell tends to be reduced, but this reduction is resisted by the rigidity of the cell wall. However, a stage is reached when the negative pressure inside the cell causes the thin area of the wall at the apex to be pulled inwards: the thicker regions of the wall are not visibly distorted. As the top of the conidiophore caves in, so the conidium is retracted in much the same way as a missile being loaded in a catapult. This 'loading' continues as further evaporation takes place. The state of tension in the aqueous contents of the cell increases until a point is reached when rupture occurs and a gas phase suddenly appears: the evidence suggests that the gas phase is water vapour. The appearance of a gas phase releases the inward pull on the caved-in wall, which springs outwards and rounds off. Whether it is (1) the elasticity of the recoiling thin wall or (2), the rounding-off process, which is responsible for projection is not known. Discharge following rounding-off of turgid cells is known in the Entomophthorales, *Sclerospora* (Peronosporales), rust aecidiospores and other fungi (Ingold, 1953). There was certainly no indication that discharge is due to a water-squirting mechanism, as occurs in certain Phycomycetes (Ingold, 1953) and in *Nigrospora sphaerica* (Webster, 1952). If this were the mechanism, it might be expected sometimes to observe a drop attached to the conidium immediately after release: this was not so.

Further evidence that a negative pressure develops inside the conidiophore head during drying was provided by the complete evagination of immature conidia. Presumably at this stage of development the basal septum which would eventually separate the conidium from the conidiophore had not developed or was still very thin. In mature conidia this thickened septum will prevent the contents from being drawn under pressure into the conidiophore.

The side to side movements of conidiophores also may be explained according to this gas phase hypothesis. As the cylindrical lower cells loose water by evaporation there is a tendency for the walls to be distorted. Slight differences in thickness of the wall will result in distortion being more pronounced in some areas than in others: consequently there will be a bending of the conidiophore. The sudden appearance of a gas phase releases the internal tension, and the elastic walls spring back to their former shape, causing rapid recovery of the conidiophore to its original orientation.

A general similarity between this hypothetical mechanism and that in the annulus cells of fern sporangia is evident.

It is of particular interest to note some observations by Ingold (1956). When viable ascospores of *Sordaria fimicola* and other fungi were allowed to dry, a small gas bubble appeared and then rapidly enlarged. If the spore was then flooded with water, the gas bubble soon disappeared. The gas phase was also produced by dehydrating with glycerol. Ingold (1956) does not consider that there is any biological significance in the occurrence of a gas phase in these ascospores. However, he does suggest that 'if a gas phase were suddenly to appear on drying in a spore finely perched on a conidiophore, the jolt might well be enough to discharge it into the air'. This was a close prediction of what is now known to occur in *D. torulosa*, but in this fungus it seems to be the conidiophore, and not the conidium, that provides the 'jolt'. The present observations have also provided evidence to support Ingold's (1956) suggestion that the temporary appearance of a gas phase in living cells may be a relatively common event. It would be of interest to determine whether it occurs in other species of *Deightoniella*, having similar conidiophore structure to *D. torulosa* (see Ellis, 1957).

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Morphogenetic Responses to Gibberellic Acid of a Radiation-induced Mutant Dwarf in Groundsel, *Senecio vulgaris* L.

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With two Plates and eight Figures in the Text

ABSTRACT

A dwarf mutant of *Senecio vulgaris* L. induced by gamma radiation, which differs from the wild-type by a single recessive gene, grew as tall as the wild-type plant, and almost five times as tall as dwarf control plants when treated at weekly intervals from the seedling stage with 10 p.p.m. gibberellic acid. The wild-type groundsel responded only slightly to similar treatment. Some evidence was obtained that the response to GA was relatively greater when plants were grown during the late autumn. Dwarfness in the mutant is due to fewer as well as to shorter internodes; the rate of leaf initiation is reduced, but the onset of the reproductive phase and the duration of the life-cycle are not affected by the mutation. Gibberellic acid leads to a conspicuous elongation of the internodes in treated dwarf plants, but not always to a significant increase in their number. An increase in the dry weight of treated plants was observed, and this was shown to be due to an increase in photosynthetic area and not to increased photosynthetic efficiency. Comparative observations of shoot morphogenesis in wild-type and dwarf groundsel, and in dwarfs treated with gibberellic acid, have shown that the type of shoot development induced by gibberellic acid in dwarf plants does not correspond exactly to the normal mode of development in wild-type plants. Increased mitotic activity in the subapical tissue of the mutant following gibberellic acid treatment resulted in greater and earlier elongation of the internodes. However, the typical form and dimensions of the wild-type plant were not wholly restored.

INTRODUCTION

FOLLOWING the discovery by Brian and Hemming (1955) that a dwarf variety of peas differing from the tall form in a single recessive gene responded more strongly to gibberellic acid (GA) than the normal tall type, and could, in fact, be brought within the height range of the tall variety by continued treatment at appropriate dosages, a number of other cases of reversal of genetic dwarfism by GA have been reported, notably the conversion of four different dwarf mutants in maize to phenotypic tall (Phinney, 1956). In all these instances, while the dwarf was highly responsive to GA, the corresponding tall forms responded little, if at all.

Not all genetic dwarfs arising by spontaneous mutation from the wild-type respond to GA, and it was thus of interest to test the response of a dwarf mutant induced in groundsel by gamma-radiation. This dwarf form of

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groundsel originated in the X_2 generation from seed exposed to 30,000 r gamma-radiation. The mutant has a very compact, rosette habit of growth, and is also conspicuously different from the normal phenotype in leaf colour. The dwarf has much darker foliage, and can be recognized without difficulty a few days after germination by this character, which distinguishes it from the wild-type before any difference in height can be detected with certainty. This mutant form of groundsel has been shown experimentally to be determined by a single recessive gene (Harland and Basford (1960) and unpublished).

There are few reports on the effects of GA on plants exposed to ionizing radiations or on their mutant progenies. Gaur and Notani (1960) observed that GA can stimulate elongation in radiation-stunted seedlings of maize. Stoy and Hagberg (1958) found that 'erectoides' mutants of barley induced by X-ray responded to GA, and observed varying degrees of extension of the rachis after treatment, and some stem elongation, but no differential response of mutant and wild-type, as in the cases of dwarf mutations, the normal varieties of barley responding as strongly to GA as the most responsive dense-ear types.

By analogy with the nutritional mutants of *Neurospora crassa* (Beadle and Tatum, 1941), and other haploid organisms, and with the thiamine mutant induced by X-ray in the crucifer *Arabidopsis thaliana* L. Heynh. (Langridge, 1955), the response of some genetic dwarfs to GA suggests that in these cases the mutant may lack the ability of the wild-type to synthesize some natural gibberellin-like substance. Whereas in chemical genetics mutational blocks are used to analyse metabolic sequences, it is the concern of developmental genetics to investigate the effects of defective and changed metabolic patterns on morphogenetic processes in mutants and experimentally induced phenocopies, and to compare them with the effects produced by the normal allele in the wild-type. Thereby it may become possible to relate gene action to specific morphogenetic processes.

It has been well documented that physiological dwarfing can, in many cases, be reversed by GA, which can evoke the effects of vernalization in some temperature-sensitive plants and photoperiodism in some light-sensitive plants. For example, Sachs, Bretz, and Lang (1959*a*, 1959*b*) investigated the morphogenetic effects of GA in rosette forms of *Hyoscyamus niger* L., and *Samolus parviflorus* Raf., and have emphasized that bolting induced by GA in these plants is identical with bolting induced by vernalization in *Hyoscyamus* and by long days in *Samolus*. It is possible that there is a common biochemical background for these types of physiological dwarfing and genetic dwarfing which are responsive to GA; a fuller investigation of the precise effects of GA on dwarf mutants may therefore be timely.

In the present work, a comparative investigation of the gross morphology and anatomy of the normal and dwarf forms of groundsel and of dwarf plants treated with GA was carried out. In order to establish how the observed differences in the mature plants were brought about during growth, shoot morphogenesis in these three types was also compared. These observations thus provide information not only about the morphogenetic effects of gibberellic acid, but also about the ontogenesis of plants of known, differing, genetic constitution, as advocated by Wardlaw (1952).

MATERIALS AND METHODS

The groundsel plants used in these investigations were X_3, X_4, and X_5 generations of homozygous tall and dwarf sibs of the X_2 family from which the mutant dwarf was recovered.

The plants used in each series of experiments were grown under glass in uniform conditions. Since it was desirable for the purposes of comparison that the plants used in each experiment should be exactly the same age, germinating seedlings were labelled on the day of emergence from the soil by placing a polythene ring round each one. Each day's germinations were marked by a differently coloured set of rings.

Plants were measured from soil level to the tip of the unexpanded leaves, or, in the case of older plants, to the base of the first formed capitulum. Trow's (1916) method of counting the number of nodes on groundsel plants was used, taking the cotyledonary node as the starting-point, or node 1, and counting the internode between this and the node on which the first single leaf is borne as internode 1.

In recording the time of the onset of flowering, anthesis was assumed when the involucral bracts of the oldest capitulum opened to expose the yellow florets of the inflorescence, and the corollas of the outermost flowers expanded.

Before weighing, plants were cut up into three separate portions, viz. roots, stems, and leaves, and dried for 48 hours in an incubator at 90° C.

Gibberellic acid was applied to small seedlings by dropping an aqueous solution on to the cotyledons and immature leaves from a pipette. Older plants were treated by spraying until all surfaces of the foliage were wetted.

For a comparative study of shoot morphogenesis in normal tall wild-type, mutant dwarf, and GA-treated dwarf groundsel, 8-day-old seedlings in the vegetative phase of development were fixed in early May 1959 for the first set of observations, and in mid-February 1960 for the second set. The materials for the 1959 investigations were fixed in 1 per cent. chromacetic fixative (Chamberlain, 1926), but more satisfactory results were obtained with Craf I solution (Sass, 1958) which was used for the 1960 series. After fixation, the materials were dehydrated in a closely graded series of ethyl alcohol—water mixtures, followed by a series of absolute alcohol—chloroform solutions, and embedded in paraffin wax. The small seedlings were orientated in the embedding wax by the cotyledons, and all were sectioned longitudinally in the same plane, through the two cotyledons, parallel to the long axis. Microtome sections were cut at 6 μ. The stems of mature plants were fixed in F.A.A. (Johansen, 1940) and transverse sections were cut at 15 μ.

All the materials were stained for 15 hours in 1 per cent. safranin, followed by Delafield's haematoxylin for 10 minutes.

Five samples of each of the three types of shoot—tall, dwarf, and GA-treated dwarf—were examined in each set of observations. Drawings were made with the aid of a camera lucida. The most median section of each shoot was traced, and the approximate location and orientation of each mitotic figure observed in the six most nearly median sections of the same shoot were plotted on this diagram. Only metaphases, anaphases, and early telophases were recorded.

OBSERVATIONS ON GROSS MORPHOLOGY

(i) *General Responses of Dwarf Groundsel to GA*

The preliminary experiments were carried out in March, April, and May 1959, when the duration of the life-cycle, from germination to seed production, was from 65 to 75 days under greenhouse conditions.

Twenty-four dwarf and twelve normal genotypes were divided into six groups of six plants; each group was grown in one 6-inch pan, and treated with 10 p.p.m. gibberellic acid as follows:

Group 1. Six dwarf seedlings were treated for the first time when they were 10 days old. The treatment was repeated six times, at weekly intervals except for one lapse of a fortnight after the third treatment.

Group 2. Six dwarf seedlings were first treated at 17 days old, and a second time a week later. No further treatments were given.

Group 3. Six normal (tall) plants were treated at 24 days old. Treatments were continued at weekly intervals until six treatments had been given.

Group 4. Six dwarf plants were treated for the first time at 31 days old, and then weekly four more times.

At the time of the final treatments the plants in groups 3 and 4 were 58 days old. The two remaining groups of six normal and six dwarf plants were the untreated controls for these experiments.

The first indication of the influence of GA on the dwarf genotypes was the change in leaf colour, which became apparent 4 days after treatment, irrespective of the stage of development at which the treatment was begun. The mutant has much darker green leaves than those of the normal plant, and this difference can be distinguished a few days after germination. After GA treatment the leaves of the dwarf became as light in colour as the leaves of normal groundsel. Brian (1959) found that the leaves of the garden pea became noticeably paler after GA treatment, and observes that this chlorosis is a general response to GA. However, the leaves of the treated dwarf groundsel were no paler than the leaves of normal, untreated tall plants, neither was any chlorotic effect observed in GA-treated normal plants, in which leaf colour was not affected.

After three weekly treatments the dwarfs in Group 1 did not differ conspicuously in height from the normal control plants of the same age. Treatment was discontinued, but after 15 days symptoms of reversion to the dwarf habit were apparent, the youngest leaves being a much darker green.

Four days after treatment was resumed these symptoms of reversion had disappeared.

The time of onset of flowering was recorded in the treated dwarfs of Group 1, and in the dwarf and normal controls; it was apparently not affected either by GA or by the mutant gene.

All the plants were measured when they were 65 days old, by which time most of them were producing fruiting heads. The mean heights in all groups of experimental plants after various treatments are summarized in Table 1. Groups of untreated tall and dwarf, and GA-treated tall and dwarf groundsel at maturity are shown in Pl. 1.

TABLE 1

The Mean Height of 65-day-old Groundsel Plants after Various Treatments with GA

Type of plant	Age at time of first treatment (days)	Number of weekly treatments	Mean height in cm.	95% confidence interval	Degrees of freedom (d.f.)
Tall (control) .	—	—	40·5	1·007	5
Tall . . .	24	6	39·7	2·724	5
Dwarf (control) .	—	—	8·3	0·189	5
Dwarf . . .	10	3+4	37·0	6·266	5
Dwarf . . .	17	2	12·6	1·419	5
Dwarf . . .	31	5	29·5	2·359	5

The height of GA-treated dwarfs was in every case significantly greater than that of the untreated controls, but it is clear that both the age at which treatment is begun and the number of treatments given are important, especially the latter. When treatment of dwarf plants was begun at 10 days old and continued to maturity they almost attained the height of normal plants (Pl. 1). Even when the first treatment was delayed for more than a month after germination the mutant was still highly responsive. The colour of the lower, most mature leaves of these late-treated plants was slightly affected but not completely converted to the characteristic pale green; a rather patchy area of lighter green was observed on the blades of these lower leaves near to the petiole.

GA treatment of 24-day-old tall plants had no effect, but it was felt that the first treatment might have been given too late for a conclusive test of the response of the wild-type, although even later treatment promoted a response in the dwarfs. It appears from this also that internode growth continues, or can continue, relatively late in ontogeny.

(ii) *Effect of GA on Plant Height*

A second series of observations was carried out during May and June 1959 when it was found that the life-cycle was much shorter than at the time of the previous experiments. Almost all the plants were fruiting at the time of the final measurements at 47 days old.

In this experiment eighteen plants were used in each group of GA-treated mutant and normal genotypes and in the two groups of controls. Plants were grown singly in pots from the seedling stage.

The plants were measured when they were 12 days old, immediately before the first application of 10 p.p.m. GA, and then weekly, at the time of each treatment, until the majority of plants in all groups were fruiting at 47 days old. The growth curves of the four groups are compared in Fig. 1.

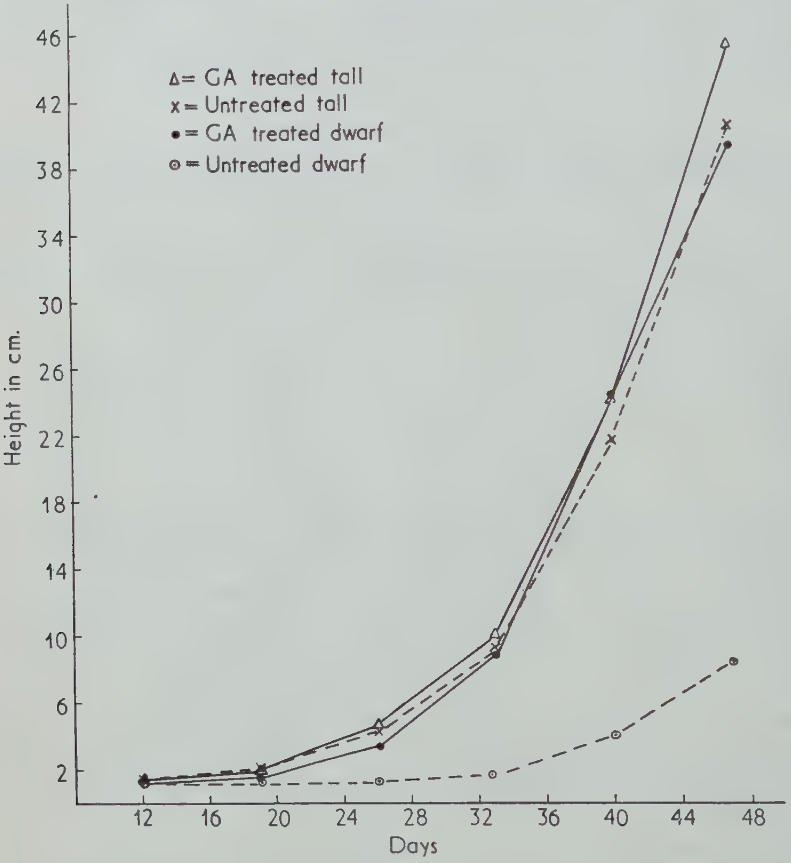

FIG. 1. The effect of GA on plant height. Each point represents the mean height of eighteen plants.

At the final measurement there was no statistically significant difference between the mean heights of the GA-treated dwarfs and the normal tall genotypes. There was, however, a difference significant at the 0·001 level between the mean height of the GA-treated normal genotypes and the control normal plants. It can therefore be said that if they receive treatment early enough, and it is maintained throughout the full period of development at intervals of one week, normal tall genotypes show a positive response to the influence of GA, although under the conditions of these experiments it is slight in comparison with the very striking response of the dwarfs.

When the mean heights of the eighteen normal and eighteen dwarf, fully mature, fruiting plants used in the second set of experiments are compared with those of the six normal and six dwarf plants at the same phase in the life-cycle used in the preliminary experiments, very little difference is observed (Table 2).

TABLE 2

Comparison of the Mean Height of Normal Tall and Dwarf Plants used in two Different Series of Experiments

Type of plant	Experimental series	Mean height in cm.	95% confidence interval	d.f.
Dwarf . .	2	8·6	1·013	17
Dwarf . .	1	8·3	0·189	5
Tall . . .	2	40·4	1·055	17
Tall . . .	1	40·5	1·007	5

Since the differences of mean heights of the dwarfs in the first set of experiments and the dwarfs in the second set, and, likewise, those of the two sets of normal plants are not statistically significant, the results obtained from the first set of experiments may be regarded as reliable although the number of plants was small.

Observations were subsequently made on the height of mature plants and untreated normal and dwarf and GA-treated dwarf groundsel grown in the greenhouse during the late autumn. The seasonal effect is very marked, as shown in Table 3.

TABLE 3

The Effect of Season on Plant Height

	Tall (normal) at maturity			GA-treated dwarfs at maturity			Untreated dwarfs at maturity		
Time of year when measured	mean height cm.	95% confidence interval	d.f.	mean height cm.	95% confidence interval	d.f.	mean height cm.	95% confidence interval	d.f.
June . . .	40·4	1·05	17	39·1	1·52	17	8·6	1·01	17
November . .	23·5	1·43	9	29·6	2·81	9	2·5	0·81	9

Warden (1935) described and contrasted winter- and summer-grown plants of *Senecio vulgaris*, and likewise found that those grown in winter were shorter in stature than those grown in the summer. He further observed that the number of internodes was approximately the same in all materials, irrespective of season.

The differences between the summer- and autumn-treated dwarf plants may not be due entirely to seasonal effects. The concentration of GA was the same in all experiments, viz. 10 p.p.m., but the autumn-grown plants were first treated on the day of emergence from the soil, and subsequently on alternate days up to the 7th day, after which they were treated once a week to maturity, when they were measured. The plants grown during May and June, on the other hand, were not treated until the 12th day after emergence from the soil, and then at weekly intervals to maturity. The autumn-grown seedlings thus received more treatments at shorter intervals during the critical vegetative phase than those grown in summer. Comparative observations of treated and untreated normal tall plants grown in winter have not so far been made, but this is clearly desirable. A greater response of tall plants to GA would indicate that the synthesis of natural gibberellin was less in winter than in summer.

(iii) *Effect of GA on Internode Number*

The number of internodes in every plant was counted at the time of the final measurements. In some cases the cotyledons were shrivelled, but they were invariably present, so that there was no difficulty in making an accurate count. The first internode above the cotyledonary node is counted as 1. The results are shown in Table 4.

TABLE 4

The Number of Internodes in Untreated and GA-treated Dwarf and Tall Groundsel Plants

Type of plant	Mean no. of internodes	95% confidence interval	d.f.
Dwarf (control) .	7·9	0·164	17
GA-treated dwarf .	8·5	0·633	17
Tall (control) . .	14·2	0·462	17
GA-treated tall .	13·8	0·586	17

All the dwarfs had fewer internodes than normal tall plants, and this comparatively low number was not significantly increased by GA treatment. It was thought, however, that this might have been because treatment was begun too late to permit the maximum effect on the apical meristem. It was probable that at the time of this experiment, towards the end of May, the 12-day-old seedlings were approaching the reproductive phase. Observations on sectioned material indicated that during May the transformation of the vegetative apex to an inflorescence apex occurs on, or very near to, the 15th day after germination (Pl. 2, Figs. 4–6). A further experiment was therefore carried out during June and July 1960. Six dwarf plants were treated with 10 p.p.m. GA on the day of emergence from the soil and on alternate days for a period of 2 weeks, and subsequently at weekly intervals. The mean number of internodes of these six dwarf genotypes was 7·5 and that of six untreated dwarf controls 7·1. These results fully confirmed those of the earlier experiments which indicated that internode number was not significantly affected by GA treatment. Treated and untreated dwarf plants had less than 60 per cent. of the mean number of internodes of the wild-type groundsel. Although the differences between the mean internode number in the GA-treated dwarfs and the control dwarfs is not statistically significant, a very slight trend towards an increase in the number of internodes was consistently observed in the GA-treated dwarfs.

Rather different results were obtained in experiments carried out by students during October and November 1960. Tall and dwarf groundsel plants were grown for 6 weeks in growth cabinets under 'daylight' fluorescent tubes, 400 f.c., with daylength of 12 hours and temperature 22±2° C. The plants were treated at weekly intervals with 10 p.p.m. GA. Under these conditions the growth habit was greatly modified, and few side branches developed. The plants did not grow vigorously, some of the mutants failing to survive. In this environment, however, treated mutants produced as many internodes on the main stem as normal talls, and the number of internodes of treated normal plants was also slightly increased.

(iv) *Effect of GA on Anthesis*

The onset of flowering occurred in both untreated and GA-treated dwarfs on the same day, but was completed a few days earlier in eighteen dwarf plants that had received GA. While there was no conclusive evidence that GA affects flowering in groundsel, it is quite certain that the mutant gene does not delay it.

(v) *Effect of GA on Leaf Size*

At the time of the final measurements of the heights of all treated and control plants used in the second series of experiments, the length and breadth of the leaf at node 6 of every plant was measured.

TABLE 5

The Mean Leaf Length and Leaf Index of the Leaf at Node 6 of GA-treated and Control Plants Measured at Maturity

No. and type of plants	Mean leaf-length	Mean leaf-index
18 Tall (controls)	124·9 (2·216)*	2·28 (0·246)
18 Tall GA-treated	125·4 (2·238)	2·48 (0·316)
18 Dwarf (controls)	54·3 (1·972)	2·48 (0.246)
18 Dwarf GA-treated	71·6 (3·758)	2·57 (0·246)

* The 95 per cent. confidence interval is shown in brackets

There is a rise and fall in the size of successive leaves on the axis of a groundsel plant, and in view of the different numbers of leaves on dwarf and tall plants, it was not easy to select comparable material. The leaf at node 6 was chosen for comparison because it was present, fully adult in form, and fresh and green on every plant. Fig. 2 shows the morphology of the leaf at the sixth node from one dwarf, one GA-treated dwarf, and one normal plant. The evident difference in leaf area will be considered in a subsequent section.

The mean leaf length and mean leaf index, i.e. length/breadth, of all four groups are given in Table 5.

The leaves of the genetic dwarf were less than half the length of those of the tall plants; and although the leaf length of the GA-treated dwarfs was significantly increased, it did not come within the range of the normal phenotype. The leaves of normal, tall genotypes were not significantly affected by GA.

Several workers have reported marked effects on leaf expansion and leaf shape following GA treatment. 'Juvenile' leaves have been observed on mature plants of *Hedera helix* (Robbins, 1957, 1960). Conversely, adult-type leaves have appeared precociously in *Eucalyptus melliodora* (Scurfield and Moore, 1958) and in *Ipomoea caerulea* (Njoku, 1958). All these modifications, which, as Allsopp (1959) has suggested, may be secondary consequences of a change in growth-rate induced by GA, have been found in plants of indeterminate growth. At the time of the first treatment at 12 days old, however, these summer-grown groundsel plants would have almost completed the vegetative phase of the life-cycle, and few, if any, leaf primordia would be initiated at the apex of the main axis after treatment. The effects described above must, therefore, have been induced in leaf primordia already present at the time of treatment.

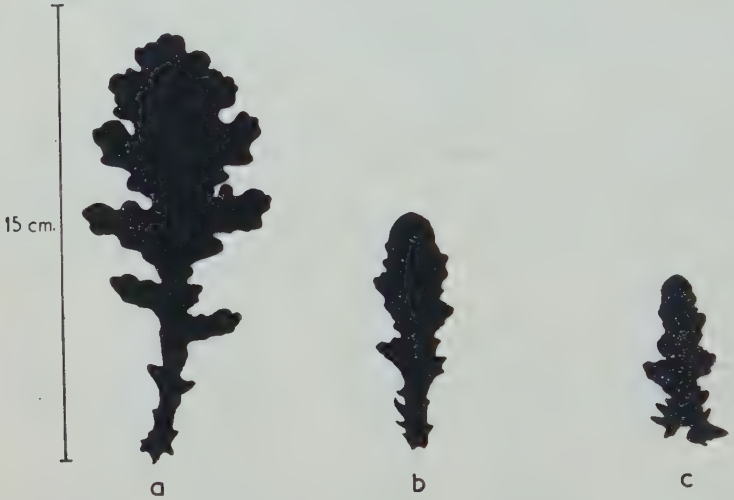

FIG. 2. The leaf at node 6 of mature plants 47-days old. *a*, untreated normal tall; *b*, GA-treated dwarf; *c*, untreated dwarf.

(vi) *Effect of GA on Dry Weight*

The dry weights of thirty normal tall and sixty dwarf seedlings were determined on the day of emergence, and statistical analysis indicated no significant difference between the mean weights of normal and dwarf plants.

Beginning on the 11th day after emergence, dry weights of the roots, stems, and leaves of ten tall plants, ten dwarfs, and ten dwarfs treated with 10 p.p.m. GA as described above (p. 285), were determined at weekly intervals over a period of 7 weeks during October and November. The 11th day after emergence was chosen as a starting-point because this was the earliest day on which dry weights of the different parts of the plants could be determined

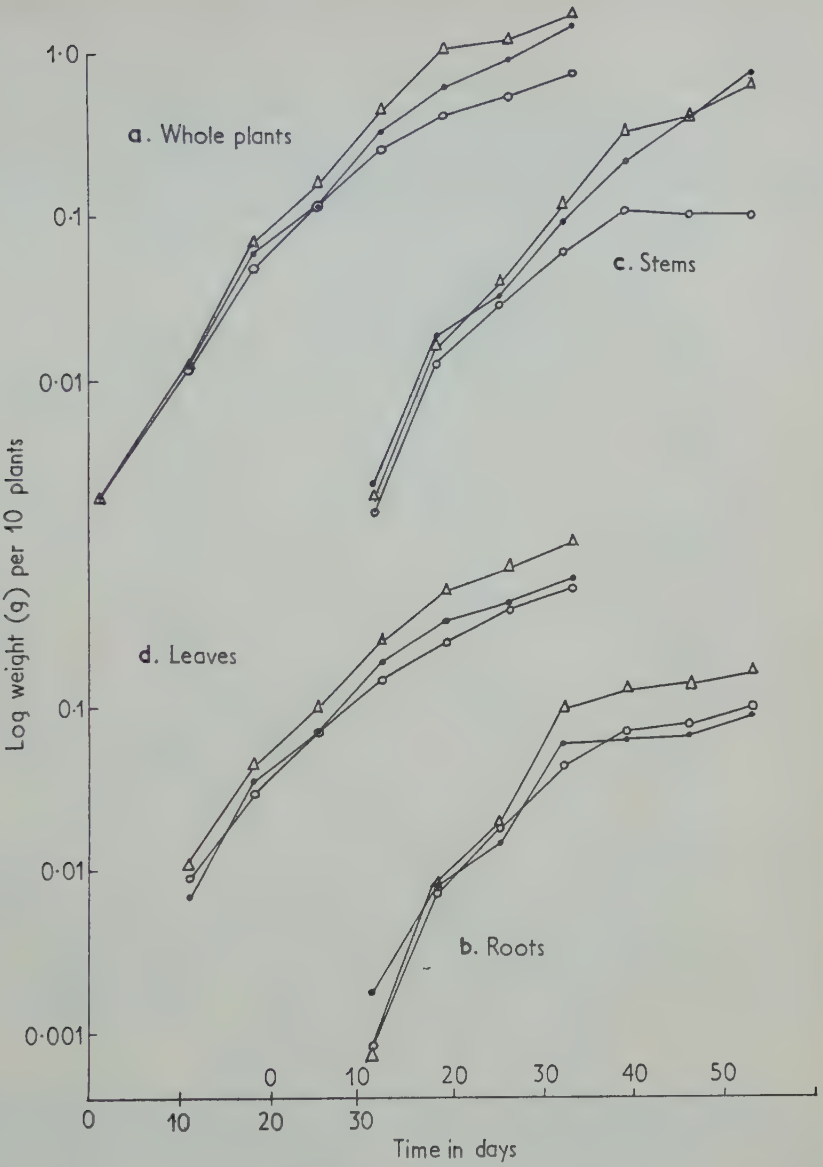

FIG. 3. The effect of GA on dry weight. *a*, entire plants; *b*, roots; *c*, leaves; *d*, stems. The graphs for stems and roots are transposed 20 days later. Symbols: ● GA-treated dwarfs; ○ Untreated dwarfs; △ Normal tall.

with accuracy in the rather small samples available, and on which leaf areas could be measured, using blue print and planimeter, for at each harvest leaf areas as well as dry weights were determined. The relative growth-rates and net assimilation rates were calculated by the methods described by Blackman and Wilson (1951).

The dry weights of treated dwarf plants were greater than those of control dwarfs at nearly every harvest, but never as great as those of normal tall plants (Fig. 3*a*). The relative growth-rates of all the plants fell as the experiment proceeded, due presumably to their increasing age, and to deteriorating light conditions as day lengths decreased (Table 6). The differences between the relative growth-rates of untreated and GA-treated dwarfs must have been due to differences in leaf areas, for net assimilation rates were similar over each harvest interval for each group of plants (Table 7). Leaf area was increased by GA-treatment (Fig. 4), thus confirming the suggestion that increase in dry weight following GA treatment is due to increased photosynthetic area and not to increased photosynthetic efficiency (Brian, 1959).

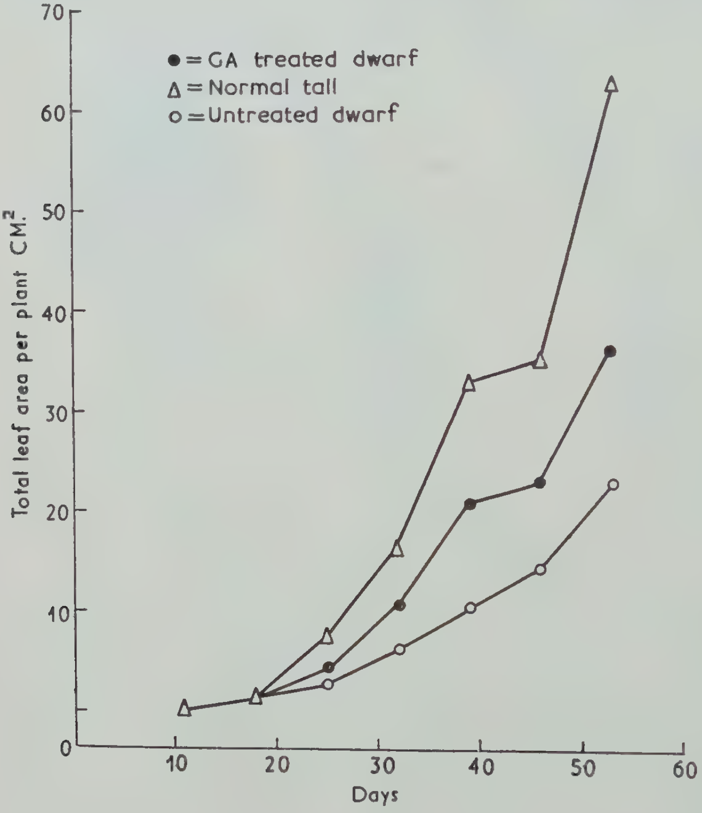

FIG. 4. The effect of GA on total leaf area.

Relatively more of the extra material that was assimilated was incorporated in stem and leaf tissue (Fig. 3 *b*, *c*, and *d*).

Dry-weight determinations in *Ipomoea caerulea* (Njoku, 1958) showed similar changes in distribution of growth of various organs as a result of GA-treatment. In this case also an increase in stem- and leaf-weights was observed; but there was little or no effect on root growth, with the result that root-weight formed a smaller proportion of total weight in treated plants.

TABLE 6

Relative Growth-rates (g./g./day) of Normal, Dwarf, and GA-treated Dwarf Plants

Harvest interval	Normal tall	Dwarf control	GA-treated dwarf
1	0·178	0·167	0·169
2	0·238	0·207	0·239
3	0·119	0·127	0·092
4	0·156	0·106	0·156
5	0·112	0·077	0·087
6	0·025	0·034	0·049
7	0·047	0·041	0·077
Mean	0·125	0·108	0·124

Required difference for significance between means ($P = 0{\cdot}05$) = 0·020

TABLE 7

Net Assimilation Rates (g./sq.m./day) of Normal Tall, Dwarf, and GA-treated Dwarf Plants

Harvest interval	Normal tall	Dwarf control	GA-treated dwarf
1	10·33	9·49	10·99
2	4·49	4·79	3·04
3	4·07	4·54	3·85
4	3·54	3·34	2·52
5	0·99	1·42	0·28
6	1·84	1·48	3·32
Mean	4·21	4·18	4·00

Required difference for significance between means ($P = 0{\cdot}05$) = 1·13

(vii) *Effect of GA on the Progeny of Treated Dwarf Plants*

Thirty-three seeds from a single fruiting head produced by a phenotypically 'tall' GA-treated dwarf genotype were sown, and twenty-seven germinated and were grown to maturity. The phenotype of all these plants was typically dwarf; the leaves were dark green, and the growth habit characteristically 'rosette'. There was no indication of a carry-over effect in the progeny of the GA-treated plant.

SHOOT MORPHOGENESIS

The conspicuous differences seen in the mature plants of normal, dwarf, and GA-treated dwarf groundsel are referable to much earlier developmental differences, and comparative observations were therefore made on shoot morphogenesis in young seedlings.

(i) *The Vegetative Shoot Apex in Groundsel*

Using the criteria of Wardlaw (1957), serial sections of fifteen vegetative apices of each of the three types of plant at the same age have been examined. Longitudinal sections show that the five outermost layers in all materials are deeply staining and unvacuolated, the nuclei being large and their nucleoli conspicuous. In the fifth (inner) layer some of the cells show evidence of differentiation, while below this layer differentiation is general. The transition to vacuolated cells is abrupt, i.e. the upper boundary of recognizable pith cells is quite distinct from the densely stained distal cells. As the apex in groundsel is somewhat flat, the level of this boundary may be used to delimit, for general descriptive convenience, the apical meristem and subapical zone.

The shape of the apex differs in the three types of plant. In the normal tall plant it consists of a low dome; in the untreated dwarf it is more conical in shape, and in the GA-treated dwarf it is typically paraboloidal, the height of the meristem above the insertion of the first leaf primordium being conspicuously greater than in the normal plant. When outline drawings of median longitudinal sections of ten apices of each type were superimposed so as to show the range in apical size and outline, the results illustrated in Fig. 5 were obtained. Serial transverse sections 6 μ thick confirm that the terminal dome of normal groundsel is relatively flat as compared with dwarf and treated dwarf. In the apex of the normal type, the youngest leaf primordium (P_1) can be seen, continuous with the apical meristem, in the first or second section of a transverse basipetal series, i.e. less than 12 μ behind the

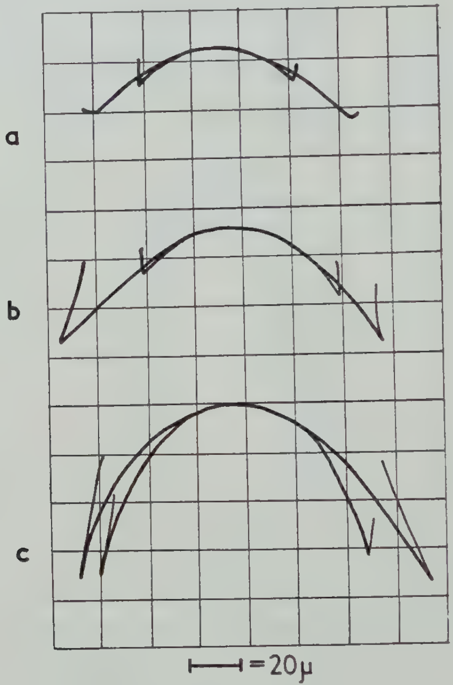

FIG. 5. The outlines of the most median longitudinal section of ten apices of each of the three types of plant at 8 days old, at the same magnification, are superimposed; only the outermost and innermost limits of the ten apical meristems are shown. *a*, normal tall; *b*, untreated dwarf; *c*, GA-treated dwarf.

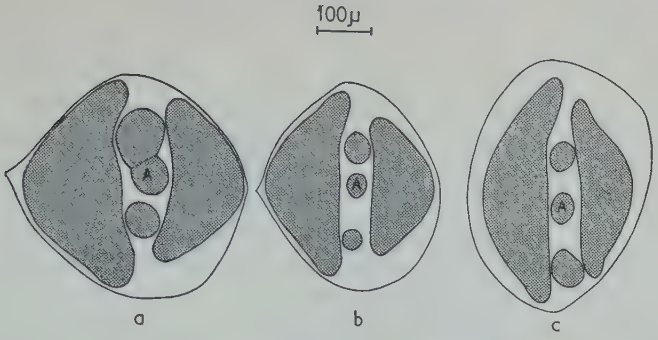

FIG. 6. Transverse sections of the tip of the shoot apical meristem of 8-day-old plants. *a*, normal tall, *b*, untreated dwarf, *c*, GA-treated dwarf. The insertion of the youngest leaf primordium, P_1, is visible in the normal seedling at this level. The inner margins of the cotyledons are shown in outline. ×70.

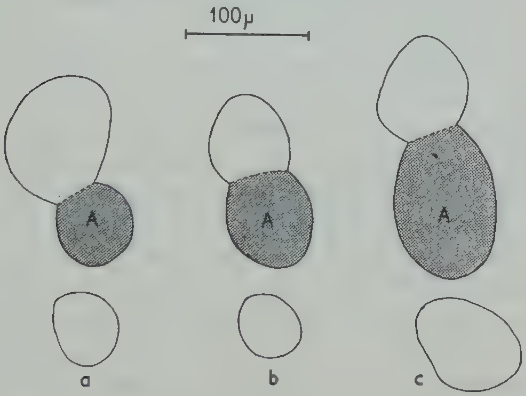

FIG. 7. Transverse sections of the shoot apex at the level of insertion of P_1. The area of the apical meristem, (*A*), of the untreated dwarf, *b*, is slightly larger, and that of the GA-treated dwarf, *c*, conspicuously larger, than that of the corresponding normal seedling at this level, *a*. ×160.

most distal cells of the apex (the depth of the central cells of the outermost layer ranges from 7–11 μ in all materials). In contrast, the insertion of P_1 is from 12–18 μ behind the most distal part of the apical mound in the untreated dwarf, and about 24 μ in the GA-treated dwarf (Fig. 6). In transverse sections at the level of attachment of P_1 in all three types, the cross-sectional area of the nascent axis of the untreated dwarf is slightly greater than that of the normal plant, while the area of the GA-treated dwarf is much greater (Fig. 7). This is a real, but as will be seen below, indirect effect of GA on the dwarf apex.

The differences in shape of the vegetative apices of the normal plant and of the untreated dwarf mutant are genetically determined, while the differences between apices of untreated and treated dwarfs are due to the effects of GA.

It is of interest to consider how these differences can be related to the distribution of growth.

The three types of apical meristem show variations in cell size but, in seedlings of the same age in all three types, the cell size and range of variation are similar for all dimensions. There were thus no differences in cell sizes in any of these materials comparable to the differences observed in the apical meristem of diploid and tetraploid *Vinca* (Cross and Johnson, 1941), and in diploid and tetraploid maize (Randolph, Abbe, and Einset, 1944). On the other hand, in most samples, as seen in median longitudinal section, there are more cells in the subdistal region, i.e. the region subjacent to the summit of the meristem, in untreated and treated dwarfs than in normal plants, and more cells in the rib meristem in GA-treated dwarfs than in untreated controls.

The differences in shape and size of the apical meristem in the three types of groundsel are not, therefore, due to differences in cell size, but to differences in cell number in the apical meristem and to the distribution of growth in the subapical region. In the untreated dwarf the subapical region is somewhat broader than that of the normal seedling, i.e. it has a larger transverse component of growth. In contrast, in the subapical region of the treated dwarf, the vertical component of growth has been increased by GA and tends to thrust the centre of the apical meristem upwards. It thus affords a striking example of the effect of a modified distribution of growth on the shape of the apex (Wardlaw, 1952, 1953, 1957): the pith and rib-meristem apparently exert upward pressure on the surrounding tissues, and as a result the contour of the apex is modified. Wardlaw (1953) has shown that such a relationship obtains in *Echinops*, in large apices of *Dryopteris*, and in other apices.

As has been described in the previous section, dry weights of roots, stems, and leaves of all materials were determined at weekly intervals to maturity, but owing to the small size of the seedlings and to the limited number of seedlings of exactly the same age available, differences in weight between these parts were not detectable before the 11th day. While it is clear, therefore, that in later stages the effects of GA on plant height are accompanied by a change in the amount of plant material and are not merely due to a redistribution of growth, comparable information is not available for the earlier stages in morphogenesis, and the changes in apical form described above may be due wholly to a redistribution of growth rather than to any increase in total amount.

The increased number of cells in the subdistal region of the apex in dwarf genotypes is the result of an increase in the amount of anticlinal division, the number of cell layers remaining unaffected. This aspect of apical activity is probably associated with, or referable to, the different rate of leaf initiation, since it takes place in the region of the inception of growth centres, where changes in relative frequencies of anticlinal and periclinal divisions are likely to affect the rate of inception of primordia. When plants are grown under normal greenhouse conditions between February and the end of July the rate of leaf initiation in the wild-type groundsel is much higher than in untreated and GA-treated mutants. The mean number of leaves formed on the main axis below the first inflorescence was 14·2 in normal tall plants, but only 7·9 in dwarfs and 8·5 in GA-treated dwarfs (Table 4) when the experimental material was grown during May and June. The transformation of the apex from the vegetative to the reproductive phase occurs virtually simultaneously in all materials.

The genetically determined difference in meristematic activity which affects the duration of the plastochron, and the GA-induced modification of the shape and structure of the subapical region, are fundamental causes of the differences in the apical meristem of normal, untreated dwarf, and GA-treated dwarf plants, and are linked with mitotic activity in the apex, which is discussed in the next section.

In the normal groundsel the pith rib meristem produces very regular files of cells, while in the untreated dwarf comparatively short chains of pith cells are formed, and their shape and arrangement are frequently irregular. As a result of GA treatment, however, regularly arranged cells in chains, or files, much longer than any seen in normal shoots of the same age, are produced (Pl. 2, Fig. 3). Cell elongation in the 8-day-old dwarfs after application of GA was comparable with normal elongation of cells in the wild-type of the same age. The largest cells in the longitudinal dimension in the pith of GA-treated dwarfs were, however, larger than any measured in the untreated dwarf at 8 days old. These anatomical differences in the subapical region of the shoot of tall and dwarf forms of groundsel correspond rather closely to those described in long and short shoots in *Ginkgo biloba* L. (Gunckel and Wetmore, 1946), and in the Rosaceae (Rouffa and Gunckel, 1951). There was no indication of precocious secondary thickening of the cell walls in the dwarf groundsel as in the mutant dwarf *Aquilegia vulgaris* described by Anderson and Abbe (1933).

(ii) *Early Effects of GA on the Shoot Apex*

Comparative observations of treated dwarf and untreated dwarf and normal 8-day-old vegetative seedlings were made using materials treated and fixed in early May 1959 and in mid-February 1960. Both sets of observations were made on the six most median sections, cut at 6 μ, of five shoots of each of the three forms. In 1959, 10 p.p.m. GA, the concentration which had effectively converted genetic dwarf to phenotypic tall, was applied to dwarf seedlings to be treated once on the day of emergence from the soil, and again on the 7th day, 24 hours before fixation. In 1960 GA at the same concentration was applied to the experimental seedlings four times; the first time on the day of emergence, and then on alternate days up to and including the 7th day.

Metaphase, anaphase, and telophase figures in the apical meristem and the subapical region of the shoot in normal tall and in treated and untreated dwarf plants were counted. The frequency and distribution of cell divisions in the three types of shoot are analysed in Tables 8 and 9, while the approximate

TABLE 8

The Number of Mitoses in a Median Panel 36 μ in Thickness (6 median longitudinal sections, 6 μ per section) of Shoot Tips of Groundsel. 1959 Observations

		Number of mitoses		
Type of plant	Shoot no.	Apical meristem	Subapical region	Total
Normal tall	1	8	4	12
	2	6	3	9
	3	5	5	10
	4	7	4	11
	5	4	7	11
	Total	30	23	53
Dwarf	6	2	1	3
	7	3	2	5
	8	3	0	3
	9	3	2	5
	10	5	0	5
	Total	16	5	21
GA-treated Dwarf	11	2	6	8
	12	3	8	11
	13	3	4	7
	14	4	9	13
	15	4	5	9
	Total	16	32	48

TABLE 9

The Number of Mitoses in a Median Panel 36 μ in Thickness (6 median longitudinal sections, 6 μ per section) of Shoot Tips of Groundsel. 1960 Observations

			Number of mitoses		
Type of plant	Shoot no.	Fig. No.	Apical meristem	Subapical region	Total
Normal tall	1		3	9	12
	2		7	8	15
	3	8*a*	9	10	19
	4		8	5	13
	5		5	5	10
		Total	32	37	69
Dwarf	6		2	6	8
	7	8*b*	3	4	7
	8		0	1	1
	9		7	2	9
	10		7	0	7
		Total	19	13	32
GA-treated Dwarf	11		0	13	13
	12	8*c*	5	28	33
	13		4	18	22
	14		3	15	20
	15		2	18	18
		Total	14	92	106

location and orientation of the axis of the spindle are shown in representative diagrams (Figs. 8 *a*, *b*, *c*). References to the Figures are given in Table 9.

In both sets of observations mitotic activity was conspicuously greater in the apical meristem and subapical region of the shoot in normal plants than in untreated dwarfs. There was no evidence that the most distal tissue of the shoot apex of the treated dwarf seedlings responded to GA, but the response of the subapical tissue is very striking in both sets of experiments, and particularly in those of 1960 (Table 9), when GA-treatment was given four times to dwarf seedlings instead of twice, as in the 1959 experiments. The majority of the divisions observed in this region of the shoot were in the transverse plane (Fig. 8); consequently the new cells would contribute to stem elongation.

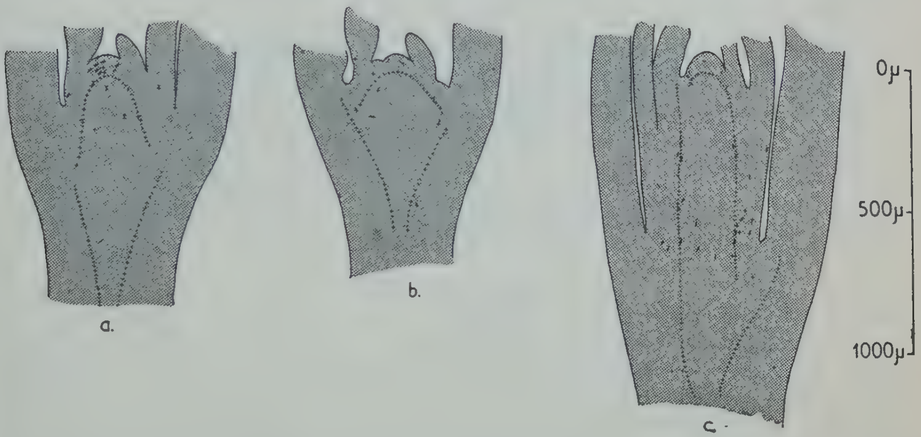

FIG. 8. The number and position of mitotic figures in a median panel 36 μ thick (6 longitudinal sections, 6 μ per section) of apices of 8-day-old seedlings. The orientation of the spindle is indicated in each case. The dotted line represents the vascular tissue—distally the prevascular tissue, and proximally the region between the xylem and phloem. *a*, normal tall, *b*, untreated dwarf, *c*, GA-treated dwarf. ×38.

Since each shoot was sectioned through the two cotyledons and therefore not necessarily through the youngest leaf primordium an accurate comparison of the frequency of cell divisions in primordia at similar stages of development was not possible; however, mitoses in young leaf primordia were counted, and there was some indication of a higher mitotic frequency in those of the normal seedlings than in those of treated and untreated dwarfs.

There was an increase in the number of cell divisions in the subapical region of all three types of shoot examined in 1960 in comparison with the number counted in corresponding material in 1959, and it was apparent that the seedlings fixed in February 1960 had made more growth in 8 days than seedlings of the same age fixed in May 1959. These differences were probably due to differences in cultural conditions. The material fixed in 1959 was grown in exceptionally warm weather, when some drying out of the soil was unavoidable. Groundsel is extremely sensitive to variations of soil moisture;

indeed Kerner and Oliver (1895) noted that groundsel growing in a dry situation in Vienna could be mistaken for another species in a wet season. Such observations, and others made in the course of this work, emphasize the importance of maintaining uniform conditions in any one experiment.

The method of assessing mitotic activity employed in this work might give misleading records if there were a difference in the duration of mitosis in the materials compared. However, the cytological observations of the shoot apex in all groundsel material considered here are consistent with the observation of gross morphology, and there is no reason to suppose that the method was inaccurate.

These observations indicate, therefore, that the effects of GA which lead to the conspicuous increase in height of dwarf groundsel plants are mediated through the response of the subapical tissues rather than those of the apical meristem itself. It may be noted also that the application of GA leads to internode elongation earlier in ontogeny than normally occurs (compare Fig. 8*a* and *c*); it has already been shown that such elongation can also continue at a relatively late stage in ontogeny.

Discussion

The results reported show that the dwarf mutant induced by gamma radiation in groundsel responds to gibberellic acid treatment in a similar manner to many dwarf plants which have arisen in other genera by spontaneous mutation from the wild-type. Tall and dwarf groundsel respond differently to GA. Dwarf plants grown in a greenhouse during the spring and summer, and treated at weekly intervals from the seedling stage with 10 p.p.m. GA, were 4·7 times as tall as the untreated mutants when both types were measured at the time of fruiting, and their mean height was not significantly different from that of normal plants of the same age. Although the normal tall genotypes are much less susceptible to GA treatment, their mean height was increased by 11·6 per cent. after six weekly applications.

It is of considerable interest that the response of normal and mutant groundsel to GA appears to be quantitatively and qualitatively different at different seasons and in different environments, and this aspect merits fuller investigation. While the height of normal and mutant plants is much less in autumn and winter than in spring and summer, the effect of GA on plant height is relatively greater. Treated dwarfs were taller than untreated normal plants at maturity when the experimental material was grown in a greenhouse during October and November.

Repeated experiments failed to induce a significant increase in the number of internodes in dwarf mutants during the normal growing season, i.e. from February to the end of summer, and these observations were consistent with the comparatively low mitotic activity in the apical meristems of both untreated and GA-treated dwarf seedlings.

An increase in the number of internodes on the main axis was, however, observed in class material grown under somewhat different environmental conditions during October and November, and such an increase must reflect the influence of GA on the apical meristem. A possible explanation for this exceptional reaction is that the synthesis of natural gibberellin is reduced during the late autumn and winter. If the actual site of gibberellin production is the apical meristem, as Lockhart (1957) has suggested, this region would not give a significant response to GA while the normal high level of natural gibberellin was maintained, as, presumably, in the ordinary growing season; but if there was a seasonal reduction in the amount of gibberellin produced, or in the rate of synthesis, the tissue would become relatively more reactive to applied GA, and might produce more leaf primordia during the protracted vegetative phase. Variations in response to GA at different seasons and in different environments may be associated with variations in the levels and ratios of growth-promoting and growth-inhibiting substances known to occur naturally in plants.

It should be emphasized that although under certain cultural conditions it was possible to induce a significantly increased number of internodes in the main axis, these treated plants were abnormal in appearance, and produced very few, poorly developed, side-branches.

In some species a conspicuous increase in the number of internodes on the main axis has been found after application of GA. Brian and Grove (1957) showed that the main axis of the 'Cupid' variety of sweet pea, which usually ceases to develop when about 6 inches high, after fourteen internodes have been produced, could be induced to develop further by GA. After seven weekly treatments with 1 μg. GA the height increased to ten times that of untreated plants, and the number of internodes increased to forty-three. Njoku (1958) observed increased leaf-production in *Ipomoea caerulea*. In *Gossypium hirsutum* Stant (1959) found that after several months' treatment with 100 p.p.m. GA, internode number, already very high in this species, was doubled. Ergle (1958), however, reported that the number of main-stalk nodes of GA-treated cotton plants was relatively unaffected. Sachs, Bretz, and Lang (1959) observed that the rate of leaf production in *Hyoscyamus* increased by as much as 70 per cent. after prolonged treatment, although in their investigations of the initial effects of GA on the development of the shoot, they found no indication of enhanced mitotic activity in the apical meristem. It seems possible that in some cases where the apical meristem does not show an immediate response to GA, a longer period of treatment, or treatment at another season, or under different environmental conditions, might induce a positive reaction.

Many dwarf plants responsive to GA, unlike groundsel, produce as many leaves on the main axis as normal plants of the same species, and at a similar rate. The genetic dwarf in *Tephrosia vogelii*, which can be converted to tall by GA, produces as many leaves as the normal form (Irvine and Freyre, 1960). Cooper (1958) found no difference in the rates of leaf initiation in the tall forms and GA-reversible dwarfs in *Lolium perenne*. The reduced stature of four different dwarf mutants of maize was related to the shorter length of the internodes, the number of internodes being essentially the same as in normal plants (Abbe, 1936). Stein (1955) studied rates of leaf initiation in two dwarf varieties of maize, brachytic-2 and dwarf-1, and in a tall variety, and found a slightly lower rate of leaf initiation in dwarf-1, but the duration of the vegetative phase in this mutant was 48 days, 20 days longer than brachytic-2 and the tall plant, and during this extended period of growth the mutant dwarf-1 produced an average of 4·2 more leaves than the tall form. Stephens (1948) describes a dwarf mutant in maize that segregated out as a simple recessive from a strain B97. In this case the dwarfing gene increased the production of nodes and associated axillary structures. It seems probable that the expression of dwarfness in these plants is due only to the failure of the internodes to elongate, and in such cases GA-induced tallness may correspond more closely to that produced by the wild-type allele.

Trow (1916) observed a positive correlation between low nodal number and early flowering in the strains of groundsel he studied. No such correlation, however, exists in the materials considered here. Initiation of the reproductive phase, anthesis, and fruiting occurred virtually simultaneously in tall plants and dwarfs, although the former habitually have about twice as many nodes as the mutants. Neither GA nor the mutant gene had any effect on the duration of the life-cycle.

The most striking early effect of GA on the dwarf seedling is to promote cell division in the subapical region. The rate of cell extension is also stimulated, and pith cell length is increased to that of the normal tall seedlings of the same age. However, although the height and dry weight of dwarf groundsel was increased up to or beyond that of the normal plant by GA treatment, an exact phenocopy of the wild-type was not produced; and the GA effect does not accurately simulate the normal mode of shoot development.

Unless the natural gibberellin produced by normal tall groundsel is highly specific so that it cannot be wholly replaced in the dwarf by the form of GA applied, the response of the mutant groundsel to GA indicates that inhibition of GA synthesis is only one effect of the gene. As a result of the mutation mitotic activity is reduced in all the meristematic tissue of the shoot, and thus the rate of leaf initiation, leaf-size, the number of nodes on the main stem prior to flowering, and the elongation of the internodes are all affected. Not all of these pleiotropic effects are modified to the same extent by GA, and the dwarf groundsel cannot, therefore, be described simply as a gibberellin-requiring mutant. However, although the study of gene action at the morphogenetic level did not, in this case, trace a direct relationship between the gene and its primary effect, it has exposed relationships between developmental processes which could not have been detected by other methods of analysis.

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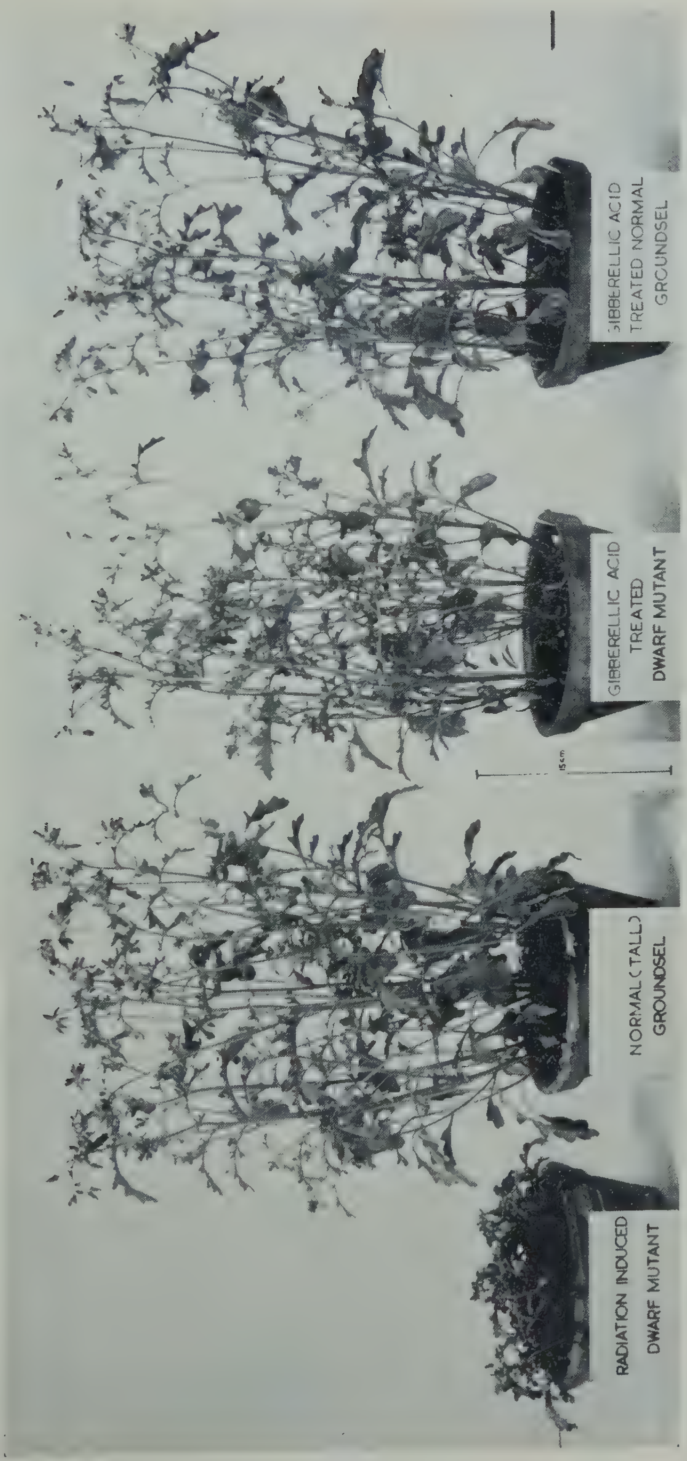
EXPLANATION OF PLATES

PLATE 1

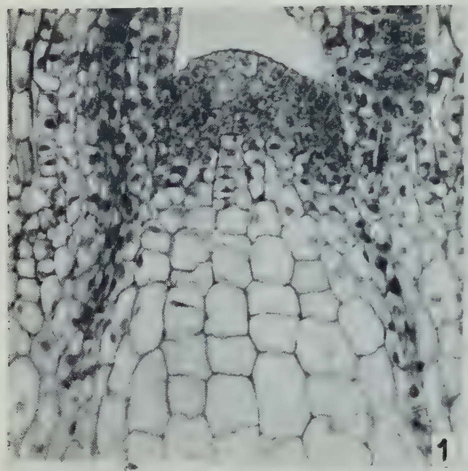
Groundsel plants sown in March at 65 days old. Six dwarf seedlings were treated for the first time at 10 days old. The treatment was repeated six times at weekly intervals, except for one lapse of a fortnight after the third treatment. Six normal, tall seedlings were first treated when 24 days old, then at weekly intervals for 5 weeks.

PLATE 2

FIG. 1. L.S. Vegetative apex of normal tall groundsel 8 days old.
FIG. 2. L.S. Vegetative apex of untreated dwarf groundsel 8 days old.
FIG. 3. L.S. Vegetative apex of GA-treated dwarf groundsel 8 days old.
FIG. 4. L.S. Inflorescence apex of normal tall groundsel 15 days old.
FIG. 5. L.S. Inflorescence apex of untreated dwarf groundsel 15 days old.
FIG. 6. L.S. Inflorescence apex of GA-treated dwarf groundsel 15 days old.
all ×250


RADIATION INDUCED DWARF MUTANT
NORMAL (TALL) GROUNDSEL
15cm
GIBBERELLIC ACID TREATED DWARF MUTANT
GIBBERELLIC ACID TREATED NORMAL GROUNDSEL

K. H. BASFORD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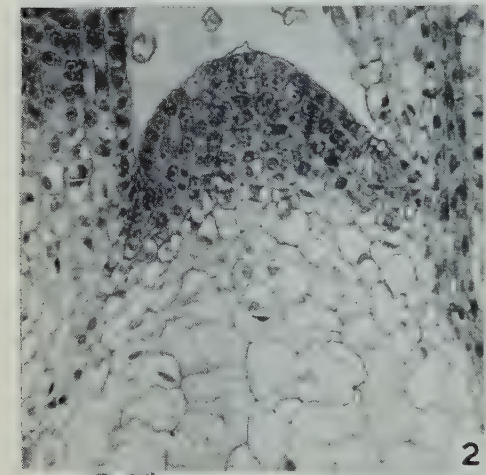
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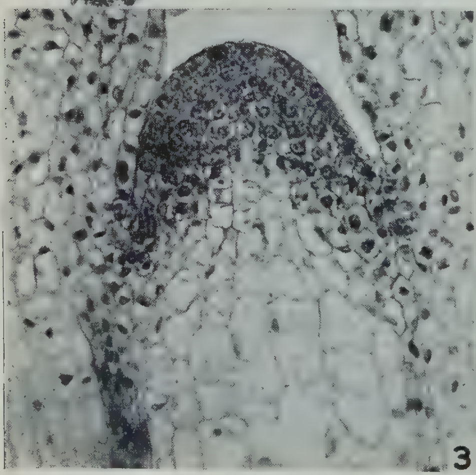
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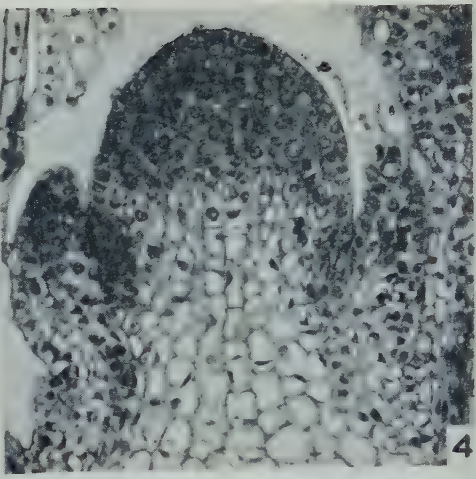
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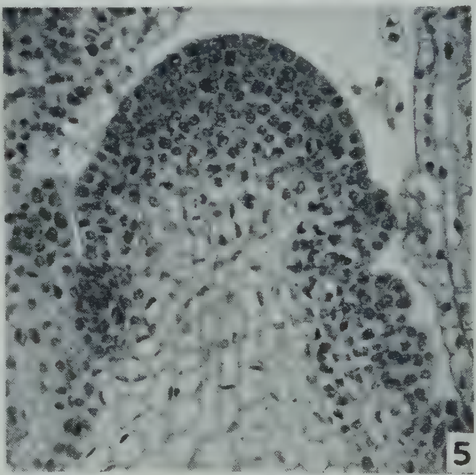
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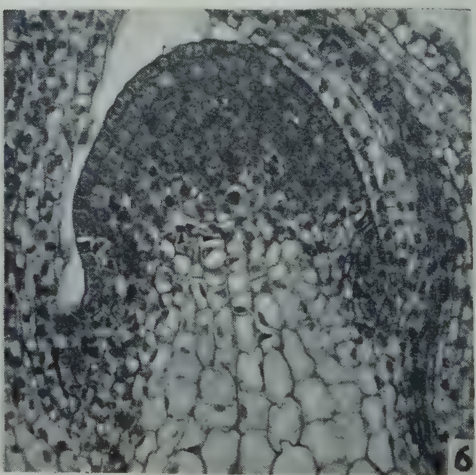
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K. H. BASFORD

The Epidermal Cell Pattern of Tetraploid *Dactylis* Cultivars

BY

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With five Figures in the Text

ABSTRACT

The length of successive leaves on the main shoot was studied in six closely related populations of *Dactylis*, and the epidermal cell pattern of the 6th leaf was examined in detail. In general, cell and blade length increased together in consecutive leaves. The cell pattern was sufficiently distinctive for the cultivars to be separable using epidermal cell length and width, in a simple replicated test.

INTRODUCTION

TWO aspects of developmental leaf anatomy have been considered in previous papers, namely, the relation between leaf and cell size in successive leaves on the main shoot in some self- and cross-fertilizing grasses (Borrill, 1959), and the value of epidermal cell pattern in taxonomic identification (Borrill, 1961*b*).

There is general agreement amongst research workers who study the developmental anatomy of *Dactylis*, that an increase in cell and leaf size occurs in the successive leaf positions on the shoot, although Zalensky (1904) reported that cell and leaf size decreased in successive leaves, in conformity with the xeromorphic trend observed in dicotyledonous plants (Borrill, 1961*a*). Leaf and cell size in six *Dactylis* cultivars have been studied to determine the morphological changes which occur in successive leaves, and at the same time to look for differences in epidermal cell pattern which may be useful in varietal identification.

MATERIAL AND METHODS

Twelve genotypes from each of the six cultivars shown in Table 1 were grown in boxes, randomized into three replicates of four genotypes each. The seeds were sown on filter-paper pads on 29/1/59, and seedlings at the same growth stage were transplanted into boxes of John Innes compost and placed in the glasshouse on 18/2/59. The length and width of successive leaves on the main shoot were measured at maturity, i.e. when half of the next leaf had appeared. The lateral tillers were cut back every 4 days. Epidermal preparations were made from a portion of the blade half-way along its length and midway between the mid-rib and margin (Clarke, 1960). Twenty cells per

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genotype were measured. The 6th leaf on all the plants and the 9th leaf on cultivars S. 143, S. 37, and Danish were studied in this way.

RESULTS

The Morphology of Successive Leaves on the Main Shoot

The trend of leaf blade length and width on the first fifteen leaves is shown in Fig. 1. The overall mean value for blade width is given, showing a progressive increase up to leaf 12, followed by a decrease. There were no significant differences between varieties for leaves of corresponding number. Blade length also shows a general tendency towards increased size which occurs at two stages, from leaf 1 to leaf 2, and again from leaves 6 to 9; there is no very obvious trend from leaves 2 to 6, or after leaf 9. The values for each cultivar are shown separately because there were differences significant

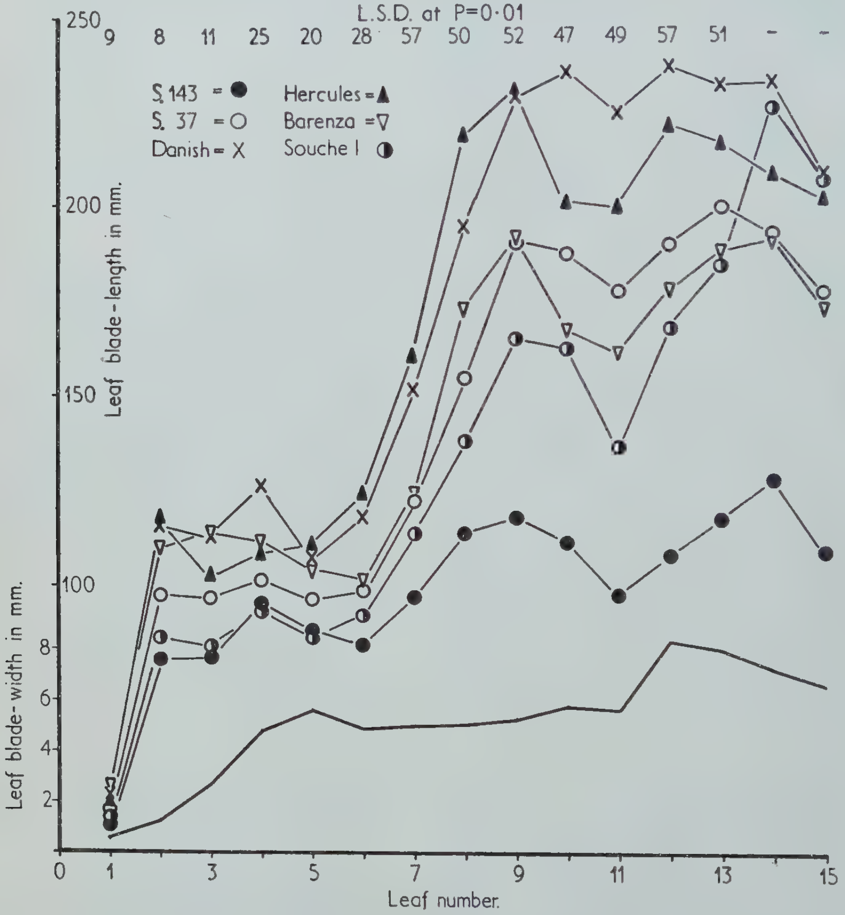

FIG. 1. Leaf blade-length in successive leaves on the main shoot in 6 *Dactylis* cultivars. Mean blade-width of the cultivars.

at the 1 per cent. level between leaves of corresponding number in the different varieties, with the exception of leaves 1, 4, and 5. The leaf blades of S. 143 were noticeably shorter than those of the other cultivars.

It has generally been supposed that low temperature and/or short photoperiods of less than 12½ hours, are a prerequisite for heading in *Dactylis*. The mean germination date was 7/2/59 in this experiment, and a 12½ hr. day was reached on 7/3/59, consequently the material received a maximum of 24 days' induction, and an inflorescence was formed on the main shoot in an average of 35·6 per cent. of the genotypes (Table 1).

TABLE 1

Heading Response of Cultivars Sown on 29/1/59 without Low-temperature Induction

Cultivar	W.P.B.S. Accession No.	Per cent. plants heading	Mean emergence date
S. 143	Bc 4812	33·0	20/5
S. 37	Bc 4806	58·0	21/5
Danish	Bc 4689	33·0	18/5
Hercules	Bc 4686	33·0	14/5
Barenza	Bc 4827	41·0	17/5
Souche I	Bc 4545	16·0	10/5
		35·6	16·6 May

Any differential effect of heading on leaf morphology, might be particularly evident in the later and larger leaves (Borrill, 1961*a*), therefore blade length in heading and non-heading plants was compared for the pooled data of leaf 9. There was no significant difference. (D/E_D 0·127.)

The relation between mean and standard deviation for the length of all the leaf blades (Fig. 2) gave a highly significant regression, indicating that the variabilities of successive leaves are similar. It seems justifiable to make comparisons between corresponding leaves of different cultivars irrespective of whether the shoots were vegetative or reproductive. It is not important which particular leaf is chosen for comparison, since increased size is accompanied by a proportionately increased variance.

Changes in cell dimensions associated with increase in blade size were studied in cultivars S. 143, S. 37, and Danish, using leaves 6 and 9 which differed considerably in size. Highly significant regressions were obtained for blade length on cell length for the 36 leaves at both positions on the shoot. In addition, the increase in mean cell size from leaf 6 to leaf 9, 43·27 to 49·13, was accompanied by an increase in length from 100 to 179 mm. (Fig. 3).

Blade width was tested against cell width, measured both at the middle and the end of the cell. No relation was observed in either case. The data thus indicate that there is a tendency towards increased blade length with leaves positioned higher on the shoot, and that larger blades are associated with increased epidermal cell length.

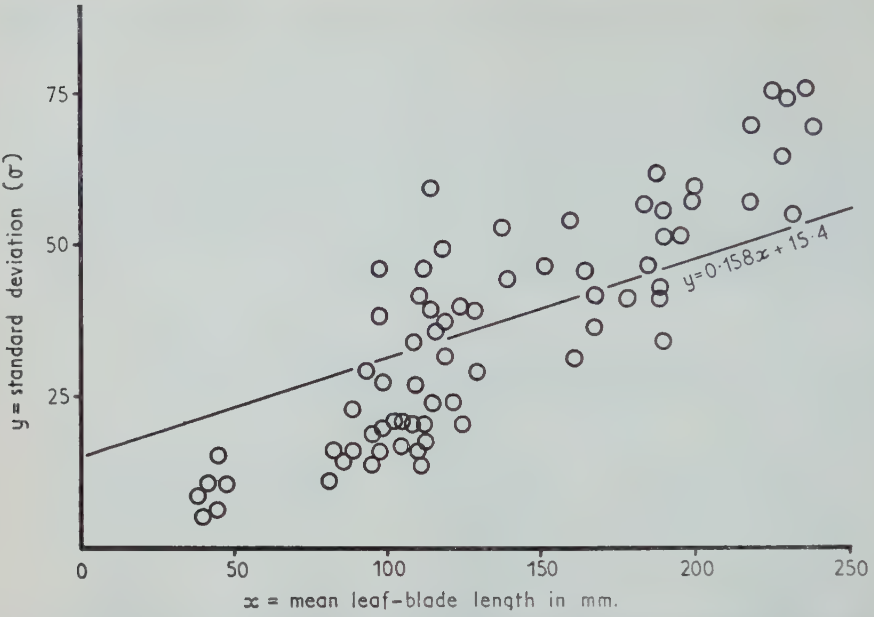

FIG. 2. The regression of standard deviation on mean blade length for leaf 6 (6 cultivars, 72 genotypes).

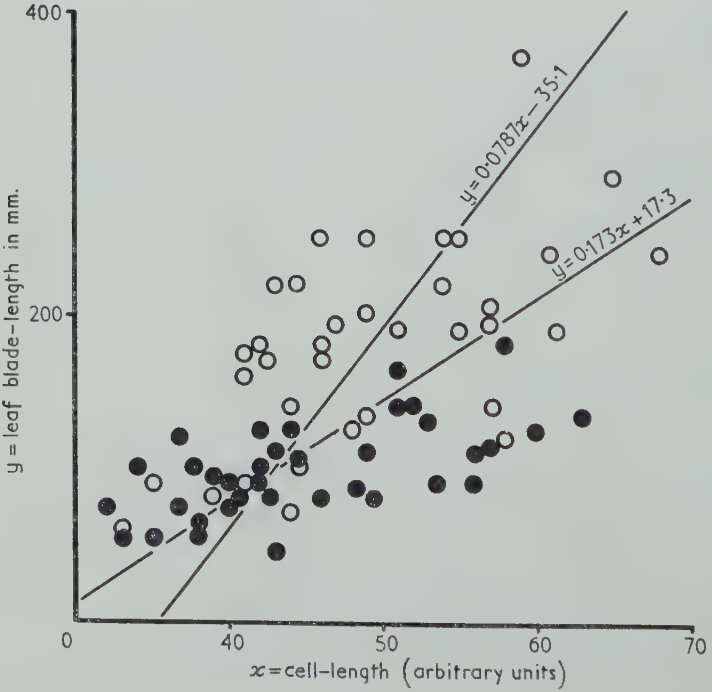

FIG. 3. The regression of leaf length on cell length, leaves 6 and 9 in six *Dactylis* cultivars.

The Epidermal Cell Pattern of the Cultivars

The 6th leaf was studied in all the varieties and the data are summarized in Table 2, and compared with measurements of the 9th leaf in cultivars S. 143, S. 37, and Danish.

The analysis of variance reveals large and significant differences between the varieties. The averages of cell length and width of the 6th leaf in each variety are shown in Fig. 5. The rectangles surrounding the points indicate the limits of variation at $P < 0{\cdot}05$. The cultivars can be separated using these characters.

Stomatal length shows no relation to any of the other features studied. A close correlation $r = +0{\cdot}71$ was found between cell size (cells per unit area) and stomatal frequency, Fig. 4. Hence in these *Dactylis* cultivars small-celled plants possess many more stomata. This confirms and extends the data obtained for diploid *Dactylis* (Borrill, 1961*b*).

Variation in cell shape can be assessed by calculating the ratios $\dfrac{\text{length}}{\text{end width}}$ and $\dfrac{\text{median width}}{\text{end width}}\cdot$ The present data, obtained from varieties of a single subspecies (D. *glomerata* subsp. *glomerata*) can be compared with previous data for several diploid subspecies (loc. cit.).

			$\frac{\text{length}}{\text{end width}}$	Diff.	$\frac{\text{median width}}{\text{end width}}$	Diff.
Subspecies glomerata	Tetraploid cultivars	Leaf 6	7·61–9·48	1·87	1·55–1·71	0·16
8 diploids	—	Random sample	5·6–20·8	15·2	1·25–1·63	0·38

The differences between the tetraploid cultivars are small compared with those occurring between the diploid subspecies. The cell shape is similar in all the varieties. There were no differences between the cultivars in type of stomatal distribution (general, or flanking the nerves), or in type of cell wall (smooth or beaded) such as characterized the diploids. Consequently identification of the cultivars on leaf characters is dependent on cell size, stomatal frequency (Figs. 5 and 4) and, to a lesser extent, on blade length (Fig. 1).

DISCUSSION

In *Dactylis* cultivars the length of successive leaf blades on the main shoot rises to a peak at about leaf 10 and then falls. In this material the main increase in length occurred between leaves 6 and 9, and was accompanied by an increase in cell length. This situation corresponds to that reported for *Glyceria*, *Lolium*, and *Triticum* (Borrill, 1959) and for *Dactylis* by Stuckey (1942).

According to Ashby (1948), Zalensky (1904) 'found that the epidermal cells in successive leaves of *Dactylis* decreased from node to node up the stem. . . . It is clear from Zalensky's data that there is a negative correlation between cell size and leaf size in the first seven leaves of *Dactylis glomerata* . . .'. It

TABLE 2

Epidermal Cell Data of the Sixth and Ninth Leaves of Tetraploid Dactylis *Cultivars (Measurements in μ)*

Cultivar	Epidermal cell data						Cell shape ratios				Stomatal length		Per unit area	
	Length		Median width		Cell-end width		Length / End width		Median width / End width				Cells	Stomata
	6	9	6	9	6	9	6	9	6	9	6	9	6	9
S. 143	181·8	198·4	41·2	35·7	24·1	22·2	7·61	9·01	1·66	1·59	48·58	49·46	75·75	49·50
S. 37	211·1	236·4	42·2	39·7	24·4	23·2	8·67	10·44	1·71	1·69	49·69	48·81	61·75	36·91
Danish	229·2	246·7	42·5	46·4	25·1	22·7	9·44	11·30	1·65	1·62	45·55	50·07	73·75	40·83
Hercules	243·1	—	44·2	—	25·8	—	9·48	—	1·70	—	47·87	—	57·16	30·41
Barenza	229·2	—	43·0	—	24·9	—	8·95	—	1·64	—	48·25	—	59·58	33·75
Souche I	228·2	—	41·5	—	26·0	—	8·77	—	1·55	—	47·88	—	57·91	36·50
L.S.D. 5%	13·1	14·9	1·00	0·66	1·00	1·04	—	—	—	—	1·10	1·04	10·06	8·60
,, 1%	15·2	27·6	2·10	0·84	1·30	1·36	—	—	—	—	1·40	1·36	13·18	1·28

does not seem possible to reconcile Zalensky's observations with those of other morphologists, since it is difficult to envisage environmental conditions under which an increase in blade length would be associated with shorter epidermal cells.

Epidermal cell patterns in the vegetative parts of the shoot are of value in the identification of grasses, as, for example, in the diploid subspecies of *Dactylis* recently studied (Borrill, 1961*b*), in which paper other examples are cited. In these groups there is a substantial accumulation of genetic differences as a result of selection in contrasting environments, and therefore the variation in cell pattern from one taxon to another is large relative to the effects of genotype/environment interaction. Consequently, replicate samples of a subspecies were consistent irrespective of environmental differences due to season or location.

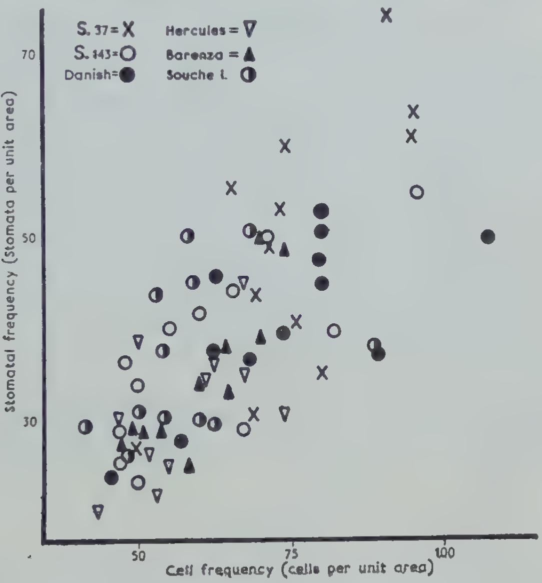

FIG. 4. Relation between stomatal frequency and cell frequency. $r = 0{\cdot}71$ (all cultivars).

The identification of individual populations such as ecotypes, or cultivars, belonging to a single taxon is more difficult because the morphological differences between them are small, and the effects of genotype/environment interaction are more likely to be of significance, making necessary a comparison of populations side by side in a randomized block, as in the present experiment. The use of seedling characters is advantageous in that screening is rapid, occupying about 7 weeks. There is no reason to keep material up to the flowering stage since there are no clear-cut differences between the inflorescences of various cultivars. These results with *Dactylis* indicate that cultivars can be separated using 20 epidermal cells from the 6th leaf of twelve genotypes from each population.

Cooper (1956) has shown that it is possible to group ryegrass (*Lolium perenne* L.) cultivars according to the effect of low-temperature pre-treatment or day-length on heading response, as measured by the number of leaves produced on the main shoot of seedlings grown in the glasshouse. This kind of test takes about 6 months. The method is so far applicable only to *Lolium* where the preconditions for heading are known to be of a clear-cut nature.

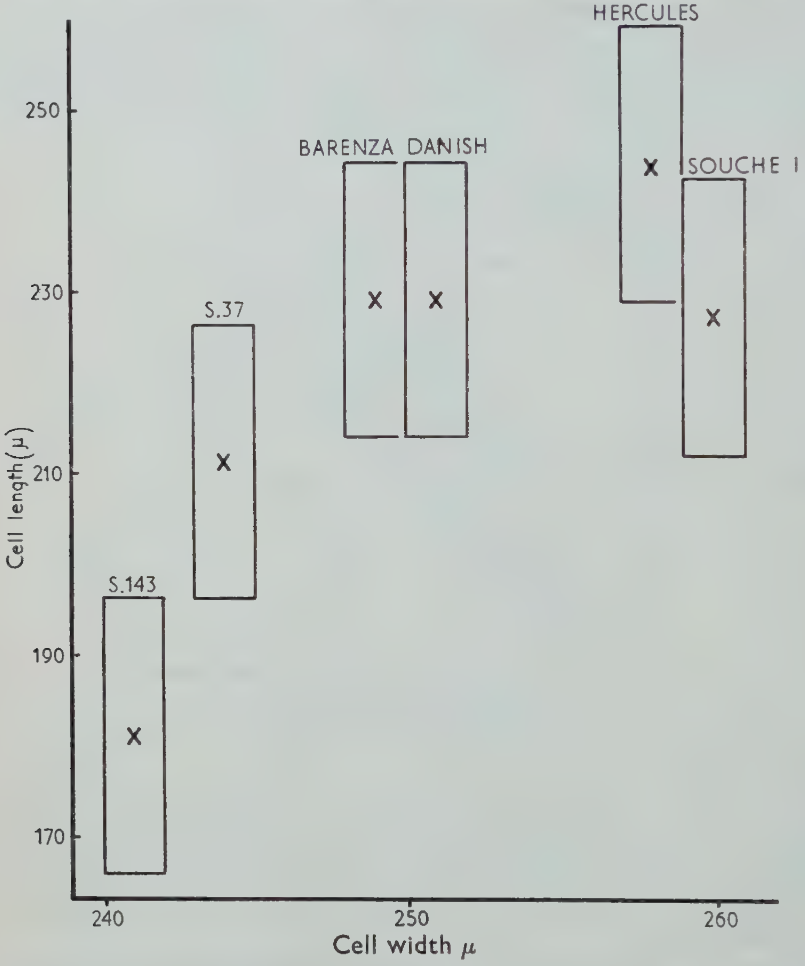

FIG. 5. Cell length and cell width in 6 cultivars of subsp. *glomerata*. Rectangles show least significant difference at $P = 0{\cdot}05$.

The separation of populations of herbage grasses on the basis of leaf anatomy or heading response is not the same as positive identification. For this, cultivars would have to be ranked in relation to the known performance of one or more varieties grown as a standard, and the environment controlled sufficiently to ensure that the order of ranking was not changed for particular varieties due to interaction with the environment.

When growing perennial ryegrass and some cereals in a glasshouse kept at between 50° and 75° F. Cooper (1957) found that the effect of variety/sowing date interaction and position in the glasshouse on heading date was small, although in some instances significant. In ryegrass, the epidermal cell pattern could profitably be studied under the same standardized glasshouse conditions, and at the same time as heading response.

Heading behaviour and cell pattern are independent methods of separating and ranking populations. Varieties identical in heading response might well differ in cell pattern, and vice versa.

The degree of stability of outbreeding cultivars may strongly influence their ease of identification. Heterozygous populations, whether wild ecotypes or bred varieties, are, in terms of succeeding generations, in a state of equilibrium with the selective forces of the environment. Management or climatic differences in the multiplication of seed stocks have been shown to lead to changes in the mean heading date of the group, although the shift may not be great (Cooper, 1959, Davies, 1954). Such changes could lead to misidentification of an unknown cultivar, and, conversely, departures from the mean of the original population in a named variety could be detected.

The separation and positive identification of closely related populations of herbage grasses is thus a difficult problem, and it is important to develop multiple screening techniques. The use of leaf epidermal cell pattern and heading response under standard conditions has proved useful, and could be developed and extended for a number of species.

SUMMARY

1. The length of the blades of successive leaves on the main shoot in six closely related *Dactylis* cultivars was found to increase to a maximum after about leaf nine and then to decrease.

2. The considerable increase in blade length from leaf 6 to leaf 9 was associated with an increase in cell length. There was no relation between blade width and cell width.

3. There were large and significant differences between the cultivars in respect of epidermal cell pattern, and the varieties were separable by using cell length and width.

4. The results are discussed in relation to developmental anatomy and varietal identification.

ACKNOWLEDGEMENTS

My thanks are due to Professor P. T. Thomas, Director, Welsh Plant Breeding Station, for his interest in this study, to Dr. J. P. Cooper for helpful discussions, and to Mr. John Clarke who made the leaf preparations.

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The Stomata of *Gnetum*

BY

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With four Figures in the Text

ABSTRACT

In both *Gnetum gnemon* and *G. ula* the stomatal development in the collars and axes of the male and female cones and the outer two envelopes of the ovule is of the haplocheilic type. In the leaf some stomata do show parallel subsidiary cells, but they arise from the surrounding epidermal cells and do not seem to have a common origin with the guard cells. This conflicts with earlier accounts and is of interest in relation to the types of stomata met with in other gymnosperms, living and fossil.

AMONG the many interesting morphological features reported in *Gnetum* one is the presence of syndetocheilic stomata in the leaves. This type of stomata was defined by Florin (1931) and is characterized by the origin of the guard and the subsidiary cells from the same mother cell. In the gymnosperms it is said to occur only in the Cycadeoidales (= Bennettitales), *Welwitschia* and *Gnetum*; all other gymnosperms show the haplocheilic type where the guard and the subsidiary cells have an independent origin, i.e. the subsidiary cells arise from the surrounding epidermal cells. It is, however, more frequent in angiosperms.

Stomata occur in *Gnetum* not only on the leaves but also on other organs like young stems, axes, and collars of the male and the female cones and the outer two envelopes of the ovule. A brief account of their development is presented here.

Preserved material of *Gnetum gnemon* was obtained from Bogor (Indonesia), Peradeniya (Ceylon), and parts of Assam (India); and of *G. ula* from Khandala, Lonavala, and Belgaum in the Western Ghats (India). The development was studied mainly from epidermal peels of young and mature leaves, cone axes, collars, and the outer two envelopes of the ovules. Portions of the above organs were warmed in water, sometimes slightly acidified with nitric acid. Each piece was then washed in water, placed on a glass slide, and the epidermis removed with the help of needles. Permanent mounts were made after staining with safranin and fast green or Delafield's hematoxylin. Microtome sections were prepared for comparison.

OBSERVATIONS

The first stomata examined by us were from the outer epidermis of the outer two envelopes of the ovule, the collars, and the cone axis. The stomatal initial or the stomatal mother cell can be recognized from the adjacent epidermal cells by its larger size, conspicuous nucleus, and dense cytoplasm

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(Fig. 1 A). The initial divides by a vertical wall into two narrow cells (Fig. 1 B) which function directly as the guard cells (Fig. 1 C). No cells of any kind are cut off from the stomatal initial before it divides to form the guard cells, and the epidermal cells around the guard cells are arranged irregularly (Figs. 1 D; 2 L–N). Thus, in the ovules and cone axes the stomatal apparatus is clearly haplocheilic and adjacent epidermal cells which happen to be little specialized have no common origin with the guard cells.

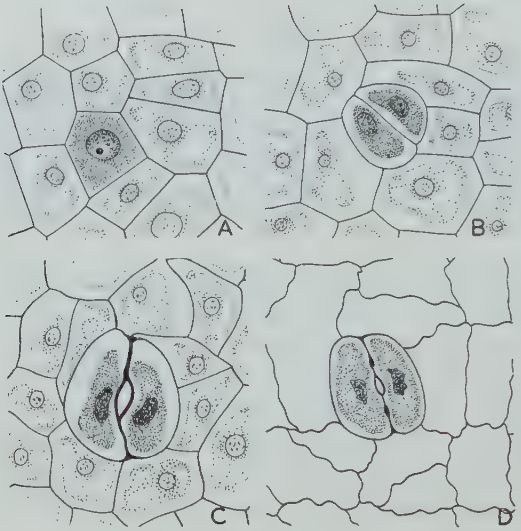

FIG. 1, *Gnetum gnemon*. A–C. Development of stomata from epidermis of outer envelope of ovule. D. Mature stoma from cone axis (All × 1,200).

According to Takeda (1913) the stomatal initials in the lower epidermis of the leaf of *G. gnemon* divide by a vertical wall resulting in the formation of two cells. One of the cells then divides similarly to give rise to a three-celled stage. In the normal course the outer two cells become the subsidiary cells while the central or guard mother cell undergoes a longitudinal division to produce the two guard cells. Thus a stoma with two subsidiary and two guard cells is said to arise from a single epidermal cell. Sometimes one or both the subsidiary cells divide longitudinally, transversely, or obliquely. This may happen even before the guard mother cell has divided. Since these observations were in disagreement with ours on the stomata from the ovules and cones of *Gnetum*, we decided to investigate the point further, using leaf material.

Epidermal peels were made from young leaves of both *G. gnemon* and *G. ula*. Stomata were absent on the upper surface, but the lower side showed abundant developmental stages. In both species the stomatal initials have dense cytoplasm and a prominent nucleus (Fig. 3 A, B). In *G. ula* the wall of the initial cell is sufficiently thickened to demarcate it from the adjacent cells

(Fig. 2 A). The surrounding epidermal cells have a vacuolated cytoplasm and may be arranged in rows (Fig. 3 B), or lie irregularly (Fig. 3 A). When the stomatal initial divides, the metaphase spindle may lie parallel to the long axis of the initial (Fig. 3 C), or at a right angle (Fig. 3 D), or in an oblique direction (Fig. 2 B). During later stages, i.e. at anaphase, the spindle fibres were invariably seen at right angles to the long axis of the initial (Figs. 2 C, D; 3 E). A longitudinal wall is next laid down resulting in two cells (Figs. 2 E–G;

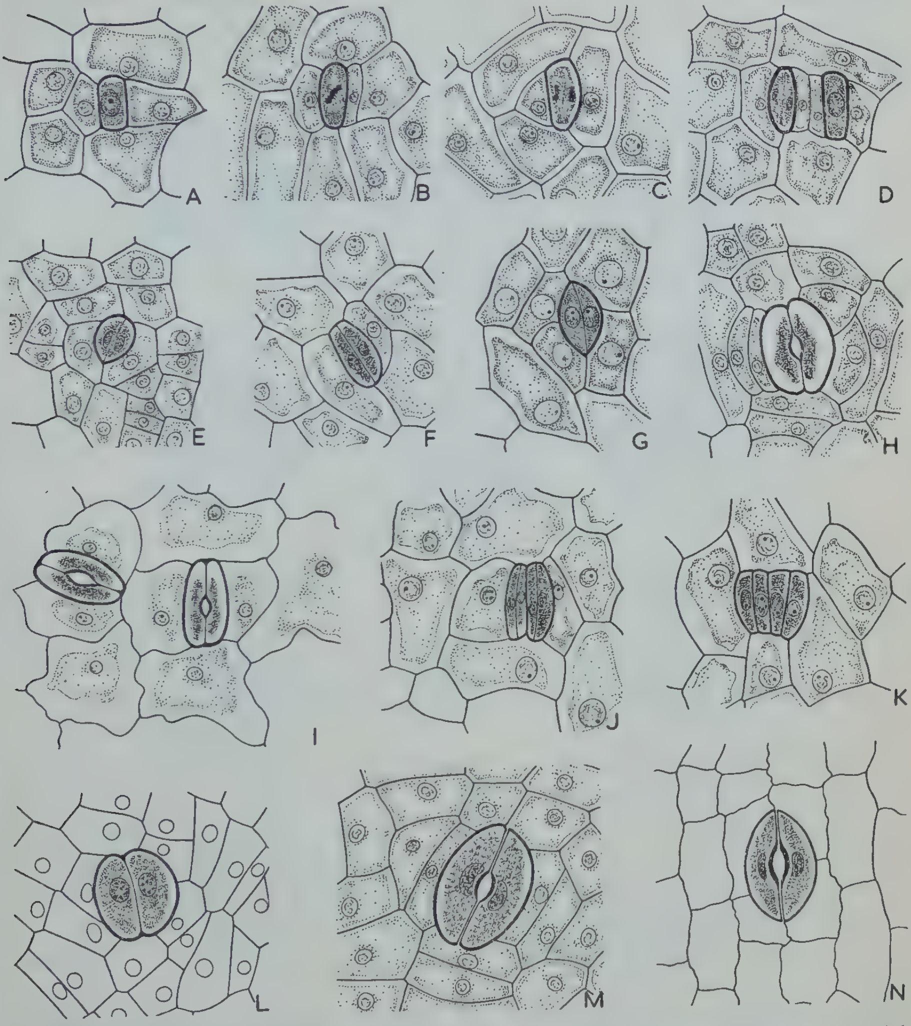

FIG. 2, *G. ula*. A. Epidermal cells from lower surface of leaf showing a stomatal initial. B, C. Stomatal initials in division. D. Two initials; the one on the left has divided. E–G. Guard cells formed from division of an initial cell. H. Stoma in which the epidermal cells encircle the guard cells. I. Mature stomata. J. Two young stomata lying adjacent to each other. K. Four prominent cells derived from two initials. L, M. Stomata from the outer envelope of an ovule. N. Stoma from female cone axis (All × 334).

3 E, F) which gradually become kidney-shaped and function directly as the guard cells (Figs. 2 H, I; 3 G). Such dividing initial cells and the two resulting guard cells were seen in a large number of preparations. In at least 50 per cent. of the stomata the epidermal cells surrounding the guard cells were arranged irregularly. Only occasionally the epidermal cells next to the guard cells lie parallel to them (Figs. 2 I; 3 H). However, it is by no means certain that these are derived by the stomatal mother cell in the manner described by Takeda. We have seen many undoubted cases of the stomatal initial dividing only once and giving rise at once to the two guard cells. Even where the cells next to the guard cells lie parallel to them, a common origin is not proved. We, therefore, regard the development of the stomata as essentially haplocheilic although some variations of pattern are possible.

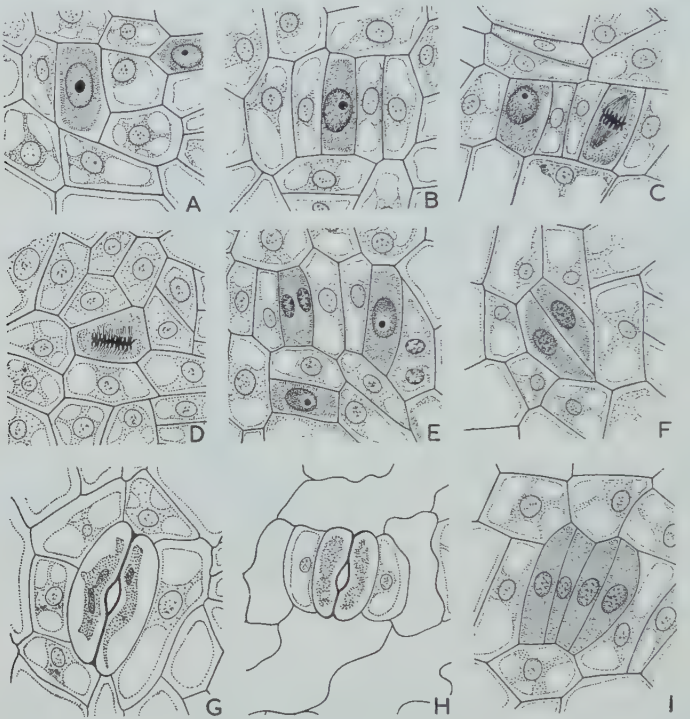

FIG. 3, *G. gnemon*. Development of stomata from lower epidermis of leaf. A–C. Stomatal initials; in C one of them is in metaphase. D. The division spindle is at right angles to the long axis of the stomatal initial. E. Three stomatal initials; one just divided. F. Newly formed guard cells. G. Older stoma. H. Stoma with subsidiary cells (is it syndetocheilic?). I. Newly formed guard cells of two adjacent stomata (H × 656; rest × 875).

Twin stomata have also been observed. These arise from stomatal initials lying side by side without any intervening cells (Figs. 2 J, K; 3 I). When these initials divide at the same time a four-celled stage results, but this is not to be mistaken for the four-celled stage of a syndetocheilic stoma.

DISCUSSION

Developmental studies on stomata are few and appear to be confined to some gymnosperms and monocotyledons. As early as 1881 Campbell studied the development of the stomata of *Tradescantia vulgaris* and *Zea mays.* A mature stoma of *Tradescantia* shows two guard cells and four specialized subsidiary cells—two lateral and two polar. In a young leaf the cells are uniform and more or less hexagonal. When a stoma is to be formed, a squarish cell is differentiated from amongst the epidermal cells. The epidermal cells on either side of this divide longitudinally to give rise to two lateral subsidiary cells. In a similar manner two polar subsidiary cells are cut off. Thus four subsidiary cells are formed, after which the stomatal initial undergoes a longitudinal division to give rise to the two guard cells. In other words, the guard cells and the subsidiary cells do not arise from the same initial. A similar situation is met with in *Zea mays* except that here the polar subsidiary cells are absent and only the two lateral subsidiary cells are present. These arise from the epidermal cells which lie on either side of the stomatal initial. At maturity they become triangular while the guard cells formed from the stomatal initial are dumbbell-shaped. Flint and Moreland (1946) have confirmed this in sugar-cane (Fig. 4 A–F). According to them the epidermal cells situated on either side of the stomatal mother cell cut off a subsidiary cell (Fig. 4 A, B). Next, the mother cell divides longitudinally and gives rise to the two guard cells (Fig. 4 C, D) which soon become bean-shaped (Fig. 4 E). The subsequent development of the stoma involves extensive modification in the shape and wall structure of the guard cells. Simultaneously with these changes in the guard cells, there is a marked increase in the size of the subsidiary cells (Fig. 4 F).

In *Phyllostachys pubescens* and *Arundinaria quadrangularis* (Fig. 4 G–L) also, investigated by Poterfield (1937), the mature stoma consists of two kidney-shaped guard cells with a subsidiary cell on either side. To all outward appearances the subsidiary cells appear to have arisen from the same initial as the guard cells. However, developmental studies prove that this is not so. The early protoderm of the culms and sheath leaves of *Arundinaria quadrangularis* is composed of small cells, mostly broader than long, having a large nucleus and dense cytoplasm. Some of these cells which can be distinguished by their specially large nuclei are the stomatal mother cells (Fig. 4 G; *smc*). The subsidiary cells of the stoma are produced by divisions cutting off lenticular segments of the protodermal cells lying next to the stomatal mother cell (Fig. 4 G–J; *sc*). The stomatal mother cell divides longitudinally after the appearance of the subsidiary cells to form the two guard cells (Fig. 4 K, L; *gc*).

Thus it is clear that in bamboo, maize, sugar-cane, and *Tradescantia* the subsidiary cells although lying parallel to the guard cells do not arise from the stomatal initial but from the surrounding epidermal cells. The appearance at maturity may sometimes be quite misleading.

Briefly, the stomata in the leaf, collars, and axes of the male and female cones and the outer two envelopes of the ovule are uniformly of the haplocheilic type in both *Gnetum gnemon* and *G. ula*. No subsidiary cells are present. In the leaf some stomata do show subsidiary cells, but as in *Phyllostachys* and *Arundinaria* they do not arise from the stomatal initial. In such fossils as the Cycadeoidales, in which syndetocheilic stomata have been described, this term can refer only to their general appearance, since no developmental studies have been made.

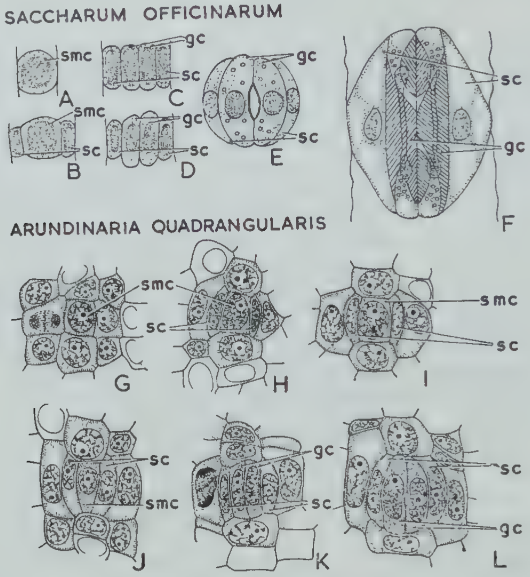

FIG. 4, *Saccharum officinarum* (A–F) and *Arundinaria quadrangularis* (G–L). Development of stomata (*gc*, guard cells; *sc*, subsidiary cells; *smc*, stomatal mother cell). A. Stomatal mother cell. B. Same with two subsidiary cells developed from adjoining epidermal cells. C, D. Stomatal mother cells divided to form two guard cells (*gc*) which are bounded on either side by a subsidiary cell (*sc*). E. Older stoma. F. Mature stoma showing two guard cells and two subsidiary cells. G. Surface view of young protoderm from the shoot of *Arundinaria quadrangularis* showing stomatal mother cell (*smc*). H–J. Stomatal mother cell flanked on either side by a subsidiary cell cut off from the epidermal cells. K. Stomatal mother cell divided to form the two guard cells. L. Mature stoma. (A–F after Flint and Moreland, 1946; G–L after Poterfield, 1937.)

We are grateful to Miss Madhu Lata for her excellent collaboration in this work, to Mr. D. M. A. Jayaweera of the Peradeniya Botanical Gardens, Ceylon, for the material of *G. gnemon*, and to Mr. D. M. Sonak for making the drawings. We had the opportunity of discussing these data, by correspondence, with Professor T. M. Harris (Reading), Professor R. Florin (Stockholm), and Dr. C. R. Metcalfe (Kew), but the responsibility for the interpretations is our own.

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Gravimorphism in Trees

1. Effects of Gravity on Growth and Apical Dominance in Fruit Trees

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With five Figures in the Text

Abstract

The effect of shoot-orientation on the vegetative growth and apical dominance of rootstocks of apple, cherry, plum, and blackcurrant was investigated. In all the species studied the total annual extension growth of horizontally grown trees was markedly less than that of vertical trees, and the effect was accentuated when the plants were rotated. In cherry and plum the reduced total growth was due both to a smaller number of internodes and also to reduced internode length; in apple and blackcurrant the difference in extension growth was mainly due to differences in internode length. In experiments with lateral shoots trained in various positions it was found that the apical dominance normally exhibited by the uppermost shoot is only manifested when it occupies a vertical position. The normal apical dominance relationships can be reversed if the uppermost shoot is grown horizontally and the second shoot vertically. Gravitational effects appear to play a role in the normal apical dominance relationships of the tree. In all species there is a marked tendency for laterals to grow out on the upper side of horizontal and arched plants.

The effects of training arched trees into various positions are considered in relation to current concepts regarding the role of auxin in apical dominance and the redistribution of auxin in horizontal organs; it is concluded that the outgrowth of laterals in arched shoots cannot be interpreted primarily in terms of these current concepts. The observed results suggest that the outgrowth of lateral buds occurs at the nearest point to the roots at which the shoot is diverted from the vertical position, and that nutrients are diverted to the highest upwardly directed meristem. It is proposed that the term *gravimorphism* should be applied to these diverse effects of gravity on plant growth.

Introduction

It has long been claimed by horticulturists that pulling down the branches of fruit trees reduces vegetative growth and promotes flowering. Thus, Champagnat (1954) claims that by arching, or by tying down a shoot horizontally, extension growth is immediately checked and that the buds show a strong tendency to initiate flowers. These effects are held to be important in the 'spindle-bush' method of growing apples (Goldschmidt and Delap,

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1950). McLean (1940) observed that looping the stems of young tree species checked their vegetative growth and promoted earlier flowering.

A few reports suggest that the orientation of the shoot may affect the normal 'correlative inhibition' of lateral buds. Thus, Vöchting (1878) found that growing branches of willow at different angles from the vertical greatly affected the outgrowth of the lateral buds, which remained dormant in a vertical shoot. Other workers observed that horizontal branches of pear and citrus produce abundant lateral shoots, which arise only on the upper side of the parent branch (Reed and Halma, 1919; Halma, 1923, 1926). It has also been observed that bending or arching branches results in the outgrowth of lateral buds, which otherwise would have remained inhibited, from the highest point of the arch (Gardner, 1925; Chandler, 1951; Champagnat, 1954).

Although there are thus a number of reports that the orientation of the shoot may affect the growth of both woody and herbaceous species in various ways, nevertheless these effects are not widely known and the possibility that such effects may be of universal occurrence has not been recognized. Moreover, no suggestions have been put forward as to the possible physiological basis of these effects, at least for woody species. It has indeed been suggested by Van Overbeek and Cruzado (1948) and Fisher (1957) that geotropic effects may be involved in the flowering responses of pineapples and soybeans, but the possibility that the position of the shoot *in relation to gravity* may be the primary factor underlying the effects of looping and pulling down the branches of fruit trees and other woody species does not appear to have been recognized. Since these effects, if established, would seem to be of considerable physiological interest and importance, they clearly merit further study. Although there are several reports in the horticultural literature of the effects of shoot orientation on growth and flowering, there appear to have been no rigorous investigations of these effects. In the present investigation, therefore, the first aim was to obtain confirmation, or otherwise, of the effects reported for fruit trees; this involved a study of the effect of shoot orientation on (1) extension growth, (2) correlative inhibition and apical dominance, and (3) flowering.

THE EFFECTS OF ORIENTATION

In this paper the effect of orientation of shoot or extension growth and correlative inhibition and apical dominance will be dealt with, while the effects on flowering will be described in a later paper. A preliminary report of these investigations has already been published (Wareing and Nasr, 1958).

1. *Effect of Gravity on Extension Growth*

Experimental. The effect of the orientation of the shoot on its growth was investigated in apple rootstock No. 3436, cherry rootstock Mazzard F12/1, plum rootstock Myrobalan B, and blackcurrant variety Baldwin, using 'maiden' trees planted in pots and restricted to a single shoot in the second year. The growth of trees maintained horizontally throughout the growing season was compared with that of the vertical controls for each species. The horizontal position was attained by placing the whole plants and their pots horizontally. One group of horizontal plants was maintained with the same side of the plant uppermost throughout the period of treatment, while the plants of a second group were rotated through 180° twice each day. The treatments were commenced shortly before bud expansion in the spring. For watering, the horizontal plants were placed erect for 1–2 minutes, as required; to eliminate possible differential effects due to rain, the soil surface of the pots of the vertical series was covered with polythene sheeting. In order to maintain the experimental material as uniform as possible, all lateral shoots were removed as they emerged. The extension growth was measured weekly throughout the growing season.

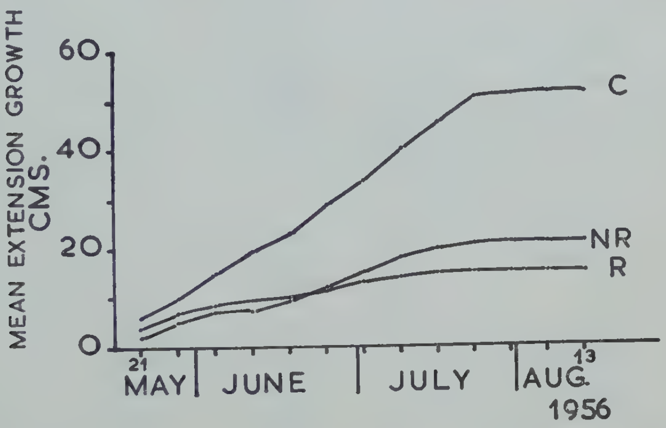

Fig. 1. Effect of shoot position on extension growth in cherry rootstock, Mazzard F12/1. C, Vertical controls. R, Horizontal, rotated. NR, Horizontal, non-rotated.

Results. By early June differences between the various experimental series became apparent, and these differences increased as the growth period progressed (Fig. 1). In all species the growth of the horizontal trees was markedly less than that of the vertical controls, and the effect of the horizontal position was accentuated when the plants were rotated. The differences at the end of the growing season were greatest in cherry and plum, where the reduced total growth of the horizontal series was due both to reduced internode length and to a smaller total number of internodes (Table 1). This smaller number of total internodes was probably partly due to the fact that extension growth ceased earlier in the horizontal plants than in the vertical (Fig. 1). In apples and blackcurrants there was less difference in the duration of extension growth of the three series, and the differences in total growth were mainly due to differences in internode length. These results were fully confirmed in various later experiments carried out during the course of this investigation.

TABLE I

Effect of Shoot Position on Extension Growth

Species and treatment	No. of plants	Mean extension growth (cm.)	Mean internode length (cm.)	Mean no. of internodes
Cherry, Mazzard F12/1 (1)				
Horizontal, rotated	16	15·4	1·2	12·4
Horizontal, non-rotated	16	21·4	1·6	13·6
Vertical, control	16	51·8	2·5	20·7
*L.S.D. for $P = 0\cdot05$	—	8·0	—	—
Apple rootstock, No. 3436 (2)				
Horizontal, rotated	19	46·9	1·4	32·0
Horizontal, non-rotated	19	52·9	1·7	31·3
Vertical, control	19	69·3	2·1	32·7
L.S.D. for $P = 0\cdot05$	—	3·9	—	—
Plum rootstock, Myrobalan 'B' (3)				
Horizontal, rotated	16	62·0	0·9	70·0
Horizontal, non-rotated	16	84·8	1·0	82·0
Vertical, control	16	116·9	1·1	102·0
L.S.D. for $P = 0\cdot05$	—	5·2	—	—
Blackcurrant, Baldwin (4)				
Horizontal, rotated	9	33·7	1·0	35·4
Horizontal, non-rotated	9	42·7	1·2	35·5
Vertical, control	9	59·0	1·7	35·1
L.S.D. for $P = 0\cdot05$	—	7·4	—	—

* Least significant difference.

2. *Interaction between Apical Dominance and Effects of Gravity*

Since lateral branches normally grow at an angle removed from the vertical, whereas the main axis normally grows vertically, it would seem possible that gravitational effects may play a part in determining the reduced growth normally made by lateral shoots as compared with the main axis. A number of experiments was conducted in 1957 and 1958 to investigate the interaction between apical dominance and the effects of gravity.

In the experiments described under this section analyses of variance, using the 't' test for significance, were carried out and the results are shown in the Appendix.

Experiment 1. This experiment was carried out in 1957 to investigate the effect of shoot orientation on apical dominance, and to study how far the growth of a lower lateral is affected by the orientation of the laterals above it.

The plant material used consisted of one-year-old stool-bed layers of the apple rootstocks MM.CIV, CVI, CIX, and CXI. The plants were pruned back to a height of 24 in., and the subsequent growth was restricted to the laterals from the uppermost three buds. The resulting lateral shoots were trained as follows:

1. All three shoots were trained into a vertical position.
2. All three shoots were trained horizontally.
3. The first two shoots were trained vertically and the third was trained horizontally.

The trees were divided into groups of three, the new growth of the trees within each group being as uniform as possible. The above treatments were applied at random for each group of trees, when the new shoots were suitable for training.

Results. The mean total extension growth for the various experimental groups is given in Table 2. The results obtained may be summarized as follows:

1. The mean extension growth of the laterals is markedly increased by training them into a vertical position as compared with the growth of the horizontally trained laterals. The differences between the three laterals were very much less where they were all trained horizontally, than where all three were vertical; this effect was particularly marked in the case of rootstock MM.CXI. Thus the normal apical dominance relationships are markedly reduced by horizontal training, but are retained where all three shoots are trained vertically.

2. The growth of the third lateral is less when trained horizontally than when trained vertically. Nevertheless, the position of the first and second lateral shoots also affects the growth of the third lateral, which is less when the first and second lateral shoots are in a vertical position.

TABLE 2

Effect of Gravity on Apical Dominance in Apple Rootstocks in 1957

			Mean extension growth (cm.)			
Treatment no.	Lateral no.	Shoot position	Rootstock MM.CXI (12 plants)	Rootstock MM.CIX (4 plants)	Rootstock MM.CIV (11 plants)	Rootstock MM.CVI (7 plants)
1	1st	V	66·2	68·5	66·7	71·2
	2nd	V	58·4	58·4	56·7	49·2
	3rd	V	50·2	44·5	44·1	37·5
		LSD*	6·3	10·9	6·6	8·3
2	1st	H	42·4	49·2	36·6	49·0
	2nd	H	40·6	44·5	34·5	41·7
	3rd	H	39·6	39·3	28·5	38·4
		LSD	6·3	10·9	6·6	8·3
3	1st	V	70·0	65·8	70·3	73·3
	2nd	V	58·3	55·5	60·0	50·1
	3rd	H	32·0	35·0	26·3	26·8
		LSD	6·3	10·9	6·6	8·3

V = Vertical. H = Horizontal.
* Least significant difference for $P = 0·05$.
For statistical treatment see Appendix I.

Experiment 2. This experiment was carried out in 1958 to investigate further the effects observed in Experiment 1, and included apples, plums, and cherries. The materials used, all maiden trees, were cut back to 30–35 buds in plums and cherries, and to about 25 buds in apples, in order to obtain them as uniform in length as possible. The growth was restricted to the distal two or three shoots according to the treatment.

The trees were trained as follows:

Series A, in which growth was restricted to three lateral shoots on each tree and the treatments being as follows (see Fig. 2):

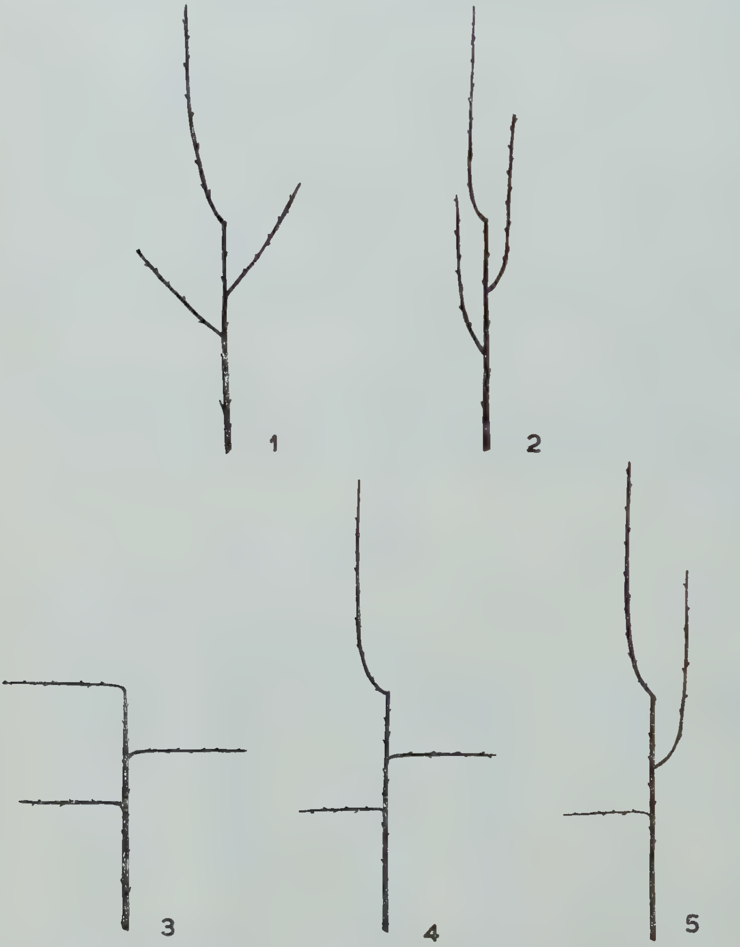

FIG. 2. Method of training plum and apple rootstocks (Experiment 2, above). The experimental data are presented in Table 3.

1. The three shoots were left in their natural vertical 'upswept' position.
2. The three shoots were trained vertically.
3. The three shoots were trained horizontally.

4. The first shoot (leader) was trained vertically and the other two shoots horizontally.
5. The first two shoots were trained vertically, and the third shoot was trained horizontally.

Series B, in which growth was restricted to two shoots, the treatments being as follows:

1. The two shoots were left in their natural vertical upswept position.
2. The two shoots were trained vertically.
3. The two shoots were trained horizontally.
4. The first shoot was trained horizontally and the second vertically.
5. The first shoot was trained vertically and the second horizontally.

In cherries the shoots were trained only as in series B, the variety used being the cherry rootstock Mazzard F12/1. With plums and apples the shoots were trained as in series A and B, the varieties used being the plum rootstock 'Brompton B' and the apple rootstock 'No. 3436'.

Results. The results obtained for the different species (Tables 3 and 4) are consistent with those obtained in the previous experiment and can be summarized as follows:

1. The growth of all the shoots is less when they are in a horizontal position as compared with the vertical, but the uppermost shoots show a proportionally greater reduction when trained horizontally than do the lower ones. In cherries and plums the growth of the shoots (whether there are two or three shoots) is almost the same when they are all trained horizontally, i.e. apical dominance is lost. In apples there are still differences in growth between shoots when they are all trained horizontally, but these differences between successive laterals are considerably reduced in the horizontal position. On the other hand, when all the shoots are trained vertically, the greater growth of the uppermost shoots results in well-marked apical dominance. Again, in this experiment, it appears that the apical dominance exhibited by the uppermost shoot is only fully manifested when it occupies a vertical position.

2. When the uppermost shoot is trained horizontally, the second shoot replaces it as a leading shoot (Table 4). This is a further evidence that the dominance of the uppermost shoot is exerted only when it occupies a vertical position.

3. When the third lateral of the plum rootstock is trained horizontally, its growth is markedly affected by the position of the first and second laterals; when the latter are trained vertically, their increased growth is accompanied by reduced growth of the third (horizontal) lateral. (Compare Treatments 3, 4, and 5, Table 3.) Similar effects were observed in apples, and although the differences were not significant in the present experiment, these differences did attain significance in two varieties of the apple rootstocks used in the preceding experiment. Similarly in the plum rootstock the growth of the leading shoot is affected by the position of the laterals below it; when the latter are trained vertically, the growth of the leading shoot is reduced as compared with

TABLE 3

Effect of Gravity on Apical Dominance, 1958

'3-shoot series'

Treatment no.	Lateral no.	Shoot position	Plums		Apples	
			No. of plants	Mean extension growth (cm.)	No. of plants	Mean extension growth (cm.)
1	1st	Untrained	6	86·6	6	71·8
	2nd	,,		63·8		64·0
	3rd	,,		53·6		51·9
		LSD*		9·0		7·0
2	1st	Trained V	7	96·5	9	71·4
	2nd	,,		85·4		59·8
	3rd	,,		71·6		51·8
		LSD		8·3		5·6
3	1st	H	10	53·4	9	55·8
	2nd	H		57·6		50·0
	3rd	H		56·6		45·1
		LSD		7·9		5·6
4	1st	V	10	102·8	10	76·5
	2nd	H		44·3		48·6
	3rd	H		42·3		39·8
		LSD		7·9		5·4
5	1st	V	9	107·7	10	72·9
	2nd	V		94·0		60·2
	3rd	H		42·7		42·9
		LSD		7·4		5·4

* Least significant difference for $P = 0{\cdot}05$.
For statistical treatment see Appendix I.

TABLE 4

Effect of Gravity on Apical Dominance, 1958

'2-shoot series'

Treatment no.	Lateral no.	Shoot position	Cherries		Plums		Apples	
			No. of plants	Mean extension growth (cm.)	No. of plants	Mean extension growth (cm.)	No. of plants	Mean extension growth (cm.)
1	1st	Untrained	5	77·2	6	93·9	6	64·3
	2nd	,,		68·9		80·5		54·9
		LSD*		7·1		10·6		6·3
2	1st	V	10	83·5	8	97·6	8	63·7
	2nd	V		69·5		82·7		51·4
		LSD		5·1		9·2		5·4
3	1st	H	10	62·9	8	59·9	8	51·8
	2nd	H		62·4		58·8		47·1
		LSD		5·1		9·2		5·4
4	1st	H	10	63·1	10	56·4	8	45·1
	2nd	V		86·1		103·6		71·3
		LSD		5·1		8·2		5·4
5	1st	V	10	80·5	10	99·7	8	69·2
	2nd	H		52·8		48·7		41·9
		LSD		5·1		8·2		5·4

* Least significant difference for $P = 0{\cdot}05$. For statistical treatment see Appendix I.

its growth when the lower laterals are trained horizontally. (Compare Treatments 2, 4, and 5, Table 3.) These results seem to indicate that there is mutual competition between the three shoots, presumably for available nutrients.

4. In plum rootstock there is a considerable stimulus of growth in the second and third laterals as a result of training them vertically, as compared with the untrained position. (Compare Treatments 1 and 2, Table 3.) This result would seem to indicate that the natural inclination of the second and third laterals from the vertical enhances the apical dominance of the leading shoot in this species. Similar effects were not observed in apple rootstock, however.

3. *Effect of Shoot Position on Outgrowth of Lateral Buds*

During the course of this investigation it was observed that there was a marked tendency in horizontal shoots for axillary buds, which normally in a vertical shoot would have remained inhibited, to grow out on the upper side (Fig. 3).

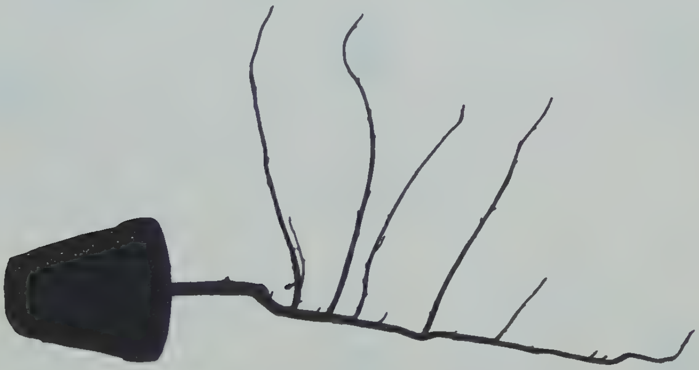

FIG. 3. Outgrowth of lateral buds from upper side of current year's shoot of blackcurrant grown horizontally.

In some species, such as blackcurrant, this outgrowth of laterals occurred even on the current year's shoot, but in apples, cherry, and Brompton plum this effect generally occurred only on the second-year wood, the buds of the current year's shoot normally remaining completely inhibited. The horticultural practices of training branches horizontally as in espaliers or pulling them downwards to form an arch, as in the spindle-bush and the 'arcure' methods of training, frequently result in the outgrowth of strong upward-growing laterals from the upper side of the parent shoot; Vöchting (1878) provided illustrations of this effect. These observations would seem to suggest that there is a loss of normal apical dominance in horizontal or arched shoots. It can be envisaged that this loss of apical dominance might be due to reduced auxin production by the shoot apical region, as a result of its horizontal or downwardly directed position. On this hypothesis the responses of the lateral buds would be determined primarily by the orientation of the apical region of the shoot. Alternatively, the outgrowth of lateral buds might be determined primarily by changes, possibly in auxin distribution, within that portion of the stem on which they are borne. Experiments were carried out to obtain evidence bearing on this problem.

Experiment I. An experiment was carried out with cherry in 1958 to determine the effect on the outgrowth of laterals, of arching the shoots in conjunction with various orientations of the shoot tip, and with girdling of the shoot at various points.

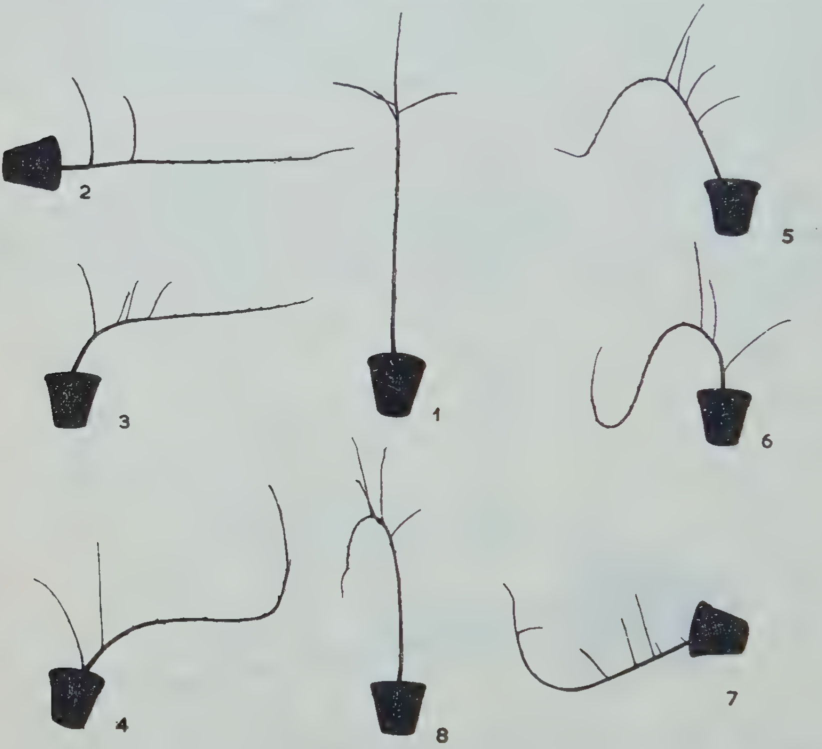

FIG. 4. Effect on outgrowth of buds of training shoots of cherry rootstock, Mazzard F12/1, into various positions. (For experimental data see Table 5.)

Maiden trees of cherry rootstock, Mazzard F12/1, planted in 7-in. pots, were trained into the various positions shown in Fig. 4, by tying them to a system of supporting wires and canes. The plants were trained well before bud-break; girdling was carried out on March 28 by removing a ring of bark about 1 cm. in width, and the callus subsequently formed was scraped off on May 19. The trees were selected to have intact terminal buds, so that the main axis was continued when these buds grew out. There were ten trees per treatment.

When growth had been completed, measurements of the extension growth of the main axis were made, and observations carried out on the outgrowth of lateral shoots; for this latter purpose the original (first-year) shoot was divided into three regions, viz. (1) the *proximal* region, between the root and the summit of the arch, (2) the *distal* region, lying between the summit of the arch and the *sub-apical region*, (3) comprising the apical few centimetres below the tip of the first-year shoot.

Results. The data are summarized in Table 5 and the results are illustrated in Fig. 4. It is seen that in the vertical 'control' shoots (Treatment 1) the outgrowth of laterals was restricted to the sub-apical region and well-marked apical dominance was exhibited by the main axis. In trees grown horizontally without arching (Treatment 2) the extension growth of the axis was markedly reduced and outgrowth of the laterals occurred not in the sub-apical region but in the basal region, as was observed repeatedly in earlier experiments.

TABLE 5

Effect of Gravity on Outgrowth of Laterals in Cherry, 1958

(10 plants per treatment)

Treatment no.	Proximal portion		Distal portion		Sub-apical region		Mean extension growth of 'leader' (cm.)
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	
1	0	—	0	—	30	79·3	35·0±1·9
2	31	67·6	0	—	2	1·9	11·6±1·6
3	28	69·8	14	28·6	0	—	13·9±2·5
4	24	57·5	3	6·5	8	15·4	22·4±2·4
5	38	99·9	0	—	5	5·4	10·2±1·6
6	35	105·8	5	10·3	2	3·2	14·8±1·3
7	35	77·2	0	—	6	7·0	17·8±2·3
8	39	108·2	7	17·2	3	5·8	12·7±1·8

(*a*) Total number of buds which grew out.
(*b*) Mean total lateral growth per plant.

Similar basal outgrowth of laterals occurred in Treatment 3, in which the trees were grown horizontally by arching in the basal region only, but there was also more development of laterals along the horizontal 'distal region', beyond the curved basal region. In Treatment 4, which differed from Treatment 3 only in that the tip region was upwardly bent, growth of laterals was still strongest in the basal region, but there was also some outgrowth of laterals in the vertical sub-apical region, although fewer lateral shoots were developed in this region than in the vertical 'controls' of Treatment 1; the extension growth of the terminal shoot was also considerably less than in Treatment 1.

In Treatment 5 the whole shoot was arched, so that the apical region was downwardly directed, but here also lateral growth was almost entirely restricted to the basal region, and the general pattern was little affected by upward bending of the apical region in Treatment 6. Essentially the same pattern was obtained in Treatment 7, in which the shoot was 'concavely' arched, so that the shoot apical region was upwardly directed.

From the results obtained with these various treatments it seems that the outgrowth of laterals, mainly from the basal region, is very little affected by the orientation of the apical region of the main axis, but seems to be determined primarily by the orientation of the remaining part of the shoot. When the basal region was maintained vertical and arching occurred in the upper part of the shoot (Treatment 8) the main zone of lateral outgrowth was shifted upwards to the arched region, thus indicating that it was not primarily proximity to the base of the shoot which determined lateral outgrowth in the preceding treatments; it would seem rather that outgrowth occurs primarily in the proximal region of any portion of the shoot which deviates from the vertical position.

Observations on the extension growth of the main axis indicated that growth was considerably reduced in all the treated trees, as compared with the vertical 'controls', even in Treatments 4 and 6 where the tips were orientated vertically, although the growth in the latter groups was greater than in the corresponding treatments (3 and 5) where the tips were horizontal. In many instances the strongest lateral from the arched shoots made greater growth than the terminal shoot of the main axis, indicating that the vertical lateral had assumed apical dominance over normal leading shoots when the latter is displaced from the vertical.

In the experiments involving girdling of the stem, the plants were arched as in Treatment 5 above, and the different girdling treatments were applied as follows:

1. Plants girdled in the proximal region (i.e. *below* the summit of the arch).
2. Plants girdled *above* the summit, a few centimetres below the terminal bud.
3. Plants girdled in both the foregoing positions.

The results of the treatments are summarized in Table 6. In the plants girdled below the summit of the arch the pattern of outgrowth of laterals was essentially the same as in Treatment 5, in which the plants were arched without girdling, but in the girdled plants the laterals which grew out below the girdle made even greater growth than in Treatment 5, the uppermost lateral being the most vigorous, which was not always the case in the ungirdled plants. There was no outgrowth of laterals on the distal side of the girdle.

In the plants girdled in the distal region the pattern of outgrowth of laterals was essentially similar to that in the ungirdled arched shoots, with the majority of laterals arising in the proximal region; a few buds also grew out in the distal region, immediately below the girdle, but the laterals here made much less extension growth than those in the proximal region.

When the plants were girdled in both positions the pattern of lateral outgrowth was similar to that in the preceding treatment, with the few buds which grew out below the distal girdle making even less growth than in the previous treatment, however.

It will be seen, therefore, that girdling the stem had very little effect on the outgrowth of laterals in arched shoots; this is a somewhat unexpected result and suggests that the normal 'correlative-inhibition' of lateral buds by the main shoot apex observed in erect shoots (presumably involving an auxin-controlled mechanism) is of little importance in determining the outgrowth of laterals in arched shoots.

TABLE 6

Effect of Arching and Girdling on Outgrowth of Laterals in Cherries, 1958

(10 plants per treatment)

Treatment no.	Proximal portion		Distal portion		Sub-apical region		Mean extension growth of 'leader' (cm.)
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	
1	27	90·2	0	—	4	3·2	7·0±2·0
2	47	139·8	0	—	10	11·6	8·6±1·1
3	39	93·1	0	—	7	4·2	

Treatments

1. Girdle in proximal region (below summit of arch).
2. Girdle in distal region (few cm. below terminal bud).
3. Girdle in both foregoing positions.

(*a*) Total number of buds which grew out.
(*b*) Mean total lateral growth per plant.

Experiment 2. The observation that it is the lateral buds in the proximal portion of arched shoots which grow out, and that there is usually no outgrowth of laterals beyond the summit of arched shoots, suggested that possibly the outgrowth of laterals may be stimulated by major nutrients or specific growth factors moving upwards from the roots. McLean (1940) observed that looping Wisteria stems resulted in dwarfed and shrubby growth. If, however, the loop was half-buried in the soil and allowed to root, normal vigorous twining plants were produced. Although McLean did not attempt to interpret his results, the latter are compatible with the hypothesis that nutrients or growth factors proceeding from the normal roots fail to reach the apical region of looped shoots, but are able to do so if roots are induced at the base of the loop. In order to confirm these observations a similar experiment was carried out with willow (*Salix viminalis*), which was chosen because of the readiness with which it can be induced to form roots.

Twenty-four uniform stem cuttings about 75 cm. in length were rooted in the spring, before extension growth had commenced. These cuttings were planted in John Innes potting compost No. 3 in boxes and 16 of them were tied into a complete loop, so that the normal shoot apical region was orientated approximately vertically. The bases of the loops of 8 plants were buried

3–5 cm. below the surface of the soil, to induce roots, which rapidly occurred. The loop in the remaining 8 plants was maintained above soil level and no adventitious rooting occurred. A further 8 plants were grown vertically as controls. The treatments are represented diagrammatically in Fig. 5.

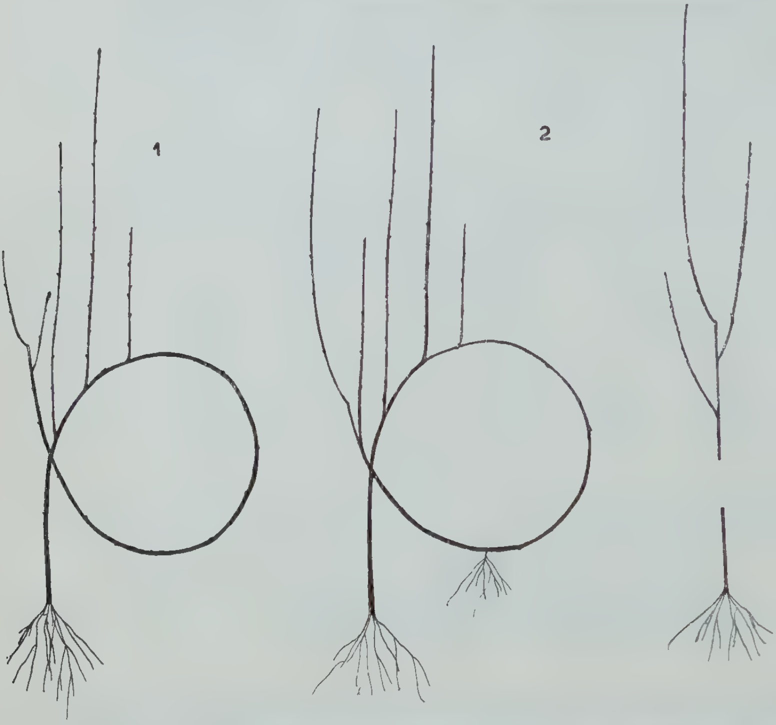

Fig. 5. Effect of looping on outgrowth of laterals of willow (*Salix viminalis*). Note that in plant no. 1 the laterals arising from the proximal part of the arch have made very strong growth, whereas those arising from the terminal region of the main axis have made greatly reduced growth compared with those in the vertical controls (3). When roots are induced at the base of the loop (2), however, the growth of the laterals arising in the terminal region is greatly stimulated.

Table 7

Effect of Looping on Growth of Willow

(8 plants per treatment)

	Proximal portion		Distal portion		Sub-apical portion		Mean extension growth of apical shoot (cm.)
Treatment	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	
(1) Non-rooted loop	5·9	693·0	1·0	43·0	1·0	30·0	39·0±6·5
(2) Rooted loop	4·9	582·5	2·1	140·8	1·6	134·0	126·0±7·0
(3) Vertical	0	—	0	—	8·5	627·3	121·0±7·5

(*a*) Mean number of buds per plant which grew out.
(*b*) Mean total lateral growth per plant (cm.).

Measurements of the extension growth of the terminal shoot and of the laterals at the end of the growth season are shown in Table 7. It is clear that looping greatly reduces the extension growth of the terminal shoot, but when the loops were rooted, extension growth was equal to that of the vertical control plants. Whereas the growth of lateral shoots was restricted to the apical region in the control plants, it was mostly confined to the proximal region of the looped plants.

DISCUSSION

It is evident from the foregoing experimental results that the position of the shoot in relation to gravity has a marked effect on its growth. In all the species studied the total annual growth of horizontal trees was markedly less than that of the vertical trees, and the effect of the horizontal position was accentuated when the plants were rotated. In cherry and plum the reduced total growth was due both to a smaller number of internodes and also to reduced internode length. In apple and blackcurrant the difference in extension growth was mainly due to differences in internode length. These results are in agreement with various reports in the horticultural literature that arching or tying down of shoots checks extension growth (Champagnat, 1954; Sternberg and Kulikova, 1957). Reduced hypocotyl extension in inverted seedlings of various species was reported by Hering (1904). On the other hand, in short-term experiments with grass nodes, Riss (1915) and Jost (1924) found *increased* extension of horizontally placed organs.

In all species there was a marked tendency for laterals to grow out on the upper side of horizontal and arched plants. This occurred on the current year's shoots in blackcurrant, but only on 2-year shoots with apple, cherry, and plum. In apple and plum the longest laterals were formed near the *base* of horizontal plants. Similar effects have been observed in other fruit trees (Reed and Halma, 1919; Halma, 1923, 1926; Champagnat, 1954).

In the series of experiments on the effects of gravity on apical dominance, it was found that the growth of all shoots is less when they are in a horizontal position as compared with the vertical, but the uppermost shoots show a greater percentage reduction when trained horizontally than do the lower shoots, so that there is little or no difference between the shoots when they are all grown horizontally. On the other hand, when all shoots are trained vertically, the greater growth of the uppermost shoots results in a well-marked apical dominance. Thus it appears that the apical dominance normally exhibited by the uppermost shoot is only manifested when it occupies a vertical position. The normal apical dominance relationships can, indeed, be reversed if the uppermost shoot is grown horizontally and the second shoot vertically. Since it was found that in plum rootstock all lateral shoots made greater growth when they were trained vertically than when allowed to assume their natural position, it would seem that gravitational effects play a role in the normal apical dominance relationships of this species. It has been proposed

(Wareing and Nasr, 1958) that the term *gravimorphism* should be applied to these diverse effects of gravity on plant growth.

It is possible that when the branches of fruit trees are forcibly constrained by pulling them down as in arching treatments, the mechanical stresses set up may be partly responsible for the reduced growth. Alternatively, growth of the downwardly directed axes of the branches may be inhibited by the strong, upwardly growing laterals which are frequently produced on branches so treated. Neither of these possible factors can have been important in the present experiments, however, since in the initial experiments the young trees were limited to a single shoot and the horizontal position was attained by placing the whole pot on its side, so that there was no bending of the shoot.

In view of the generally accepted evidence for the role of auxins in geotropic responses, it would seem probable that these 'gravimorphic' phenomena also involve hormonal disturbances arising under the influence of gravity. For the purposes of discussion the following generally accepted assumptions will be made:

1. Auxin is produced in the shoot apical region and moves predominantly in a basipetal direction by 'polar' transport.
2. The inhibition of lateral buds is due to the auxin produced in the shoot apical region, and which is transported down the stem at concentrations which are supra-optimal for bud-growth ('Direct' theory of auxin inhibition of buds; Thimann, 1937).
3. Under the influence of gravity, auxin tends to accumulate on the lower side of horizontal organs, so that there is a lateral redistribution as well as 'polar' transport.

It is difficult to explain the reduced extension growth of horizontal shoots in terms of these concepts, except by postulating supporting hypotheses, for which there is at present no evidence. The observed outgrowth of lateral buds on the upper side of horizontal and arched shoots, however, is compatible with the hypothesis that the auxin moves from the upper to the lower side, so that its concentration on the upper side is no longer supra-optimal and the buds on this side are therefore released from inhibition.

The 'classical' theory that there is differential redistribution of auxin in horizontal organs has been re-examined by several workers in recent years. Experiments involving ^{14}C-labelled indole-acetic acid failed to give evidence of differential distribution in geotropically stimulated organs (Reisener and Simon, 1960), but since this auxin is rapidly metabolized, such negative results cannot be regarded as conclusive. On the other hand, fresh evidence has been obtained that auxin is present in greater quantities on the lower sides of horizontal coleoptiles (Gillespie and Briggs, 1959). Further, Brauner and his co-workers have obtained evidence that not only is there redistribution of auxin in the tip of geotropically stimulated coleoptiles, but that there is also differential sensitivity to auxin in the upper and lower sides of the *basal* region of horizontal coleoptiles (Brauner and Hager, 1957; Brauner and Appel, 1960). These observations are in accordance with the results of the present investigation, which seem to indicate that the perception of a gravitational stimulus is not confined to the apical regions of the shoot.

The hypothesis that buds grow out on the upper side of horizontal shoots because of reduced auxin levels does not explain, however, why the outgrowth of buds is largely restricted to the proximal region of arched shoots—on the foregoing hypothesis there would seem no reason why outgrowth of lateral buds should not occur also on the upper side of the *distal* regions of such shoots. Moreover, certain other observations suggest that the outgrowth of laterals in an arched shoot is not primarily due to a reduction in the amount of auxin coming from the shoot apical region. Thus, a girdle made in the distal region of an arched shoot causes some outgrowth of laterals immediately behind it, but the majority of laterals still arise in the proximal region and make greater extension growth than those in the distal region (Table 6); if bud-inhibition is primarily due to supra-optimal concentrations of auxin in the stem, it would be expected that the buds immediately below a girdle would make the greatest growth. For these reasons it seems doubtful whether the effects observed in the present investigation can be interpreted solely in terms of current concepts regarding the 'direct' inhibition of buds by supra-optimal auxin concentrations.

The 'direct' theory of bud inhibition of Thimann (1937) has been subject to criticism in recent years; thus Jacobs (1960) has reported that when auxin is applied to decapitated shoots at physiological concentrations there is little or no inhibition of the lateral buds. It is particularly difficult to interpret certain results of the present experiments in terms of the 'direct' theory of correlative inhibition; for example, in Experiment 2 (p. 326) it was shown that if the uppermost lateral shoot is trained horizontally and the lower one vertically, normal apical dominance relations are reversed. Similar effects were observed in arched trees, where frequently a lateral shoot was found to make greater growth than the main axis. In such cases it seems unlikely that the reduced growth of the 'leading' shoots, which normally would be 'dominant', was caused by supra-optimal auxin concentrations coming from the strongest laterals; in any case, such a hypothesis would require predominantly acropetal transport of auxin over considerable distances, which has never been demonstrated. Such effects observed in the present work would seem to be more compatible with 'indirect' theories of the role of auxin in apical dominance and correlative inhibition. Various theories for such 'indirect' action of auxin have been put forward; thus, Went (1939) suggested that the topmost bud, by virtue of its high auxin content, somehow diverts to itself either major nutrients or some accessory growth factor. This hypothesis is difficult to apply to the present results, if it is assumed that there is, in fact, *less* auxin on the upper side of arched shoots.

Several other workers have stressed the importance of nutrient distribution in correlative inhibition. Loeb (1924) held that bud inhibition is due to the monopolization of growth materials by the dominant growing areas. Gregory and Veale (1957) also hold that nutrition is the main factor in correlative inhibition.

These nutritional theories do not, as they stand, explain the effects of gravity on apical dominance observed in the present experiments, however; these observations strongly suggest that outgrowth of lateral buds occurs at the nearest point to the roots at which the shoot is diverted from the vertical position. This conclusion is supported by the fact that if a shoot is arched near the base, then strong shoots arise on this basal curved region; if, however, the shoot is arched at a higher level, so that the base of the shoot is vertical, then the buds in the basal region remain inhibited, and the outgrowth of buds occurs higher on the shoot, again on the proximal side of the arch (Fig. 4 (8)). These and other observations strongly suggest that *some mechanism is involved whereby nutrients are diverted to the highest upwardly directed meristem; other factors being equal, proximity of laterals to the roots appears to confer an advantage*, as shown by the fact that in horizontally grown trees it is the most basal lateral shoots which make the greatest growth (Fig. 2). These 'rules' would seem to hold not only for the outgrowth of lateral buds from arched to horizontal shoots, but also for the apical dominance relations observed in the experiments in which the laterals growing from decapitated rootstocks were trained in various ways (Experiments 1 and 2, pp. 324–9.)

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APPENDIX

1. *Interaction between Apical Dominance and Effects of Gravity*

(*Experiment 1, p. 324*)

Combined analysis of variance on all 4 varieties of apple rootstocks. (Data of Table 2, p. 325.)

Component	D.F.	Variance	V.R.	P
Total	305	—	—	—
Plant	101	—	—	—
Variety	3	305·4	1·98	N.S.
Type	2	8028·2	52·1	< 0·1%
Type/Variety	6	265·9	1·7	N.S.
Error (*a*)	90	153·9	—	—
Lateral	2	14271·4	236·0	< 0·1%
Lateral/type	4	2641·7	43·0	< 0·1%
Lateral/variety	6	61·0	1·0	N.S.
Lateral/type/variety	12	124·8	2·0	5%
Error (*b*)	180	60·4	—	—

2. *Analysis of Data of Table 3 (p. 328)*

Plums

Component	D.F.	Variance	V.R.	P
Total	125	—	—	—
Plant	41	544·4	—	—
Type	4	3851·8	20·0	< 0·1%
Error (*a*)	37	186·8	—	—
Lateral	2	14031·3	230·8	< 0·1%
Lateral/type	8	2796·2	45·9	< 0·1%
Error (*b*)	74	60·8	—	—

Apples

Component	D.F.	Variance	V.R.	P
Total	131	—	—	—
Plant	43	187·2	—	—
Type	4	639·5	4·5	< 1%
Error (*a*)	39	140·9	—	—
Lateral	2	6303·4	172·2	< 0·1%
Lateral/type	8	331·1	9·0	< 0·1%
Error (*b*)	78	36·6	—	—

3. *Analysis of Data of Table 4 (p. 328)—Experiment 2, 1958*

Cherries

Component	D.F.	Variance	V.R.	P
Total	89	—	—	—
Plant	44	200·2	—	—
Type	4	662·2	4·3	< 1%
Error (*a*)	40	154·0	—	—
Lateral	1	602·7	18·9	< 0·1%
Lateral/type	4	1762·8	55·3	< 0·1%
Error (*b*)	40	31·9	—	—

Plums

Component	D.F.	Variance	V.R.	P
Total	83	—	—	—
Plant	41	—	—	—
Type	4	2321·8	15·0	< 0·1%
Error (*a*)	37	154·4	—	—
Lateral	1	719·3	8·4	< 1%
Lateral/type	4	6207·6	72·9	< 0·1%
Error (*b*)	37	85·2	—	—

Apples

Component	D.F.	Variance	V.R.	P
Total	75	—	—	—
Plant	37	—	—	—
Type	4	240·6	1·79	N.S.
Error (*a*)	33	134·1	—	—
Lateral	1	533·7	17·96	< 0·1%
Lateral/type	4	1535·8	51·7	< 0·1%
Error (*b*)	33	29·7	—	—

Growth of Hyphal Branching Systems in *Coprinus disseminatus*

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With five Figures in the Text

ABSTRACT

Apical portions of hyphal branching systems of *Coprinus disseminatus* were observed at the margins of colonies extending at a constant rate under one set of environmental conditions. Growth was consistently monopodial, the main hyphae continuing to extend in the colony margin at a constant rate. Primary branch initiation occurred at regular intervals, closely associated with cell division of the parent hypha, and initiation of secondary branches took place in the older parts of the system. Extension rates of main, primary branch, and secondary branch hyphae were distinct, being in the proportions 100:66:18. In the majority of systems successive branches of the same category closely resembled one another and changes in extension rate of primary branches were small from soon after initiation up to a length of 750μ. In a minority of systems branch behaviour was less regular, some branches increasing in extension rate whilst others decreased. Changes in older parts of the systems were not followed.

Young primary branch hyphae were consistently narrower than main hyphae. Although extension rate was correlated with hyphal diameter for main and for branch hyphae separately, the extension rate of equally wide hyphae was significantly higher for main as compared with branch hyphae. Furthermore the wider, faster-extending branch hyphae tended to arise from the wider, faster-extending main hyphae.

The significance of these findings is discussed.

INTRODUCTION

It is clear from morphological studies, e.g. Corner (1948), that fungal structures are formed by the growth, differentiation, and interaction of a single fundamental unit of construction, the hyphal branching system. This system consists essentially of an apically extending filament which sooner or later branches to form further apically extending and branching filaments (Reinhardt, 1892). The experimental study of fungal growth in terms of this unit of construction has received comparatively little attention in the past but it is considered that such an analysis will be helpful towards a fuller understanding of fungal morphogenesis. Growth problems in terms of the hyphal branching system involve not only the factors affecting the growth of an isolated hypha but also the possibilities of interaction both within the branching system, e.g. branch initiation, and between separate systems.

Growth of single hyphal branching systems from germinating spores of

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Botrytis cinerea has been described by Smith (1924). He was, however, unable to follow more than the early stages of growth since the mycelium quickly ramified and became organized into colonies. Ryan, Beadle, and Tatum (1943) have drawn attention to the collective properties of hyphae in a colony. Usually a colony has a sharply defined outer margin at right angles to the direction of growth of the regular array of more or less parallel hyphal apices. Thus in Petri-dishes colonies from a point inoculum are usually circular in outline and remain so throughout colony extension. Inside the extending margin of juvenile hyphae there may be further growth (Plomley, 1959) and differentiation.

On a nutrient agar medium the rate of colony extension by many fungi at first increases with time but soon reaches and maintains a constant level (Cochrane, 1958). In *Neurospora crassa* manipulation of various external factors, e.g. temperature, pH of the medium and nutrient concentration, affected the rate of growth but not its constancy with time (Ryan, Beadle, and Tatum, 1943). A similar linear extension rate has been reported for growth of *Merulius lacrymans* through a non-nutrient medium from a food-base (Butler, 1957). Thus after an initial lag period marginal extension of such a colony appears to represent some sort of steady state (Emerson, 1950). Observations on the mycelium of *M. lacrymans* suggested that this reflected an orderly monopodial growth of marginal hyphae (Butler, 1958). In this paper an analysis is made of the dynamics of growth of individual hyphal branching systems in the margins of colonies of *Coprinus disseminatus* growing under such apparent steady-state conditions. It was thought this analysis would help to define more clearly problems of mycelial growth in terms of individual hyphal branching systems and thus provide a basis for experimental work.

MATERIALS AND METHODS

The investigation was carried out on dicaryotic mycelium of *Coprinus disseminatus* (Pers. ex Fr.) S. F. Gray (synonym *Psathyrella disseminata* (Pers. ex Fr., 1821) Quél.) derived initially from a single hyphal tip. The fungus was grown in 9-cm. Petri-dishes each containing 20 ml. distilled water agar (2 per cent. Difco agar) from a central inoculum disc 10 mm. in diameter and 3 mm. high. Inoculum discs were taken from just inside the margin of colonies growing on 3 per cent. malt agar, or on 3 per cent. malt agar supplemented with 0·12 per cent. sodium nitrate. Preliminary experiments showed that under these conditions after the 2nd day from inoculation the rate of extension of the colony margin was constant, was lower than that on nutrient agar but was higher than that on water agar from a water-agar inoculum (Fig. 1). This medium was chosen because the marginal hyphal density was low and thus the origins of most of the hyphae could be seen clearly. Hyphae growing on the agar surface in the margins of colonies were observed directly under a microscope. The Petri-dish, without lid, was placed on the stage of the microscope, the whole being enclosed in a moist chamber at a relative humidity just below saturation. The first observations were made at room temperature (mean 20° C., range 17–23° C.) but subsequently the apparatus was maintained at a constant temperature of 21·5° C. Growth of branching systems (using 2/3 inch objective) and of individual hyphae (using 1/6 inch objective) was recorded by taking serial photomicrographs at regular time intervals. The resulting 35-mm. negatives were projected on to a screen on which hyphal measurements were made.

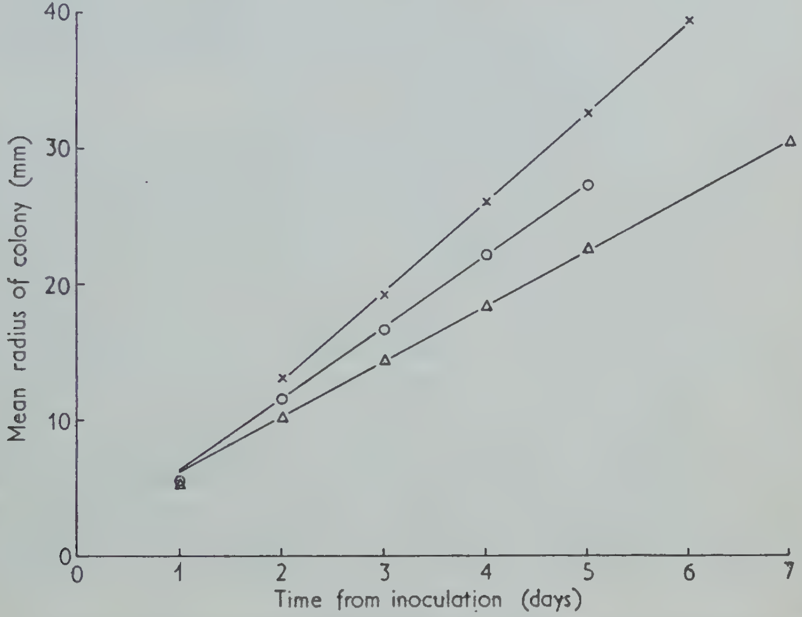

FIG. 1. Radial extension of colonies of *C. disseminatus* under different nutritional conditions at 22·5° C. The nutrient agar contained glucose (0·2 %), $NaNO_3$ (0·024 %), and a basal mineral and vitamin solution (Valder, 1958) diluted to 1/5. × malt inoculum on nutrient agar; ○ malt inoculum on water agar; Δ water inoculum on water agar.

RESULTS

1. *Growth of the Hyphal Branching System*

Serial photomicrographs of twelve individual systems were taken at 15-minute intervals for periods of *c.* 3 hours and the rate of extension of every hyphal tip was measured. The part of each system which was followed consisted of a leading hypha of the mycelial margin and all branches arising from it within *c.* 1000 μ of the tip at the start of observations. The experiment was carried out at room temperature. Results from three individual systems are shown on Fig. 2. Certain general features of growth were evident from an analysis of these systems.

(*a*) *Growth of main hyphae.* The parent hypha of each system grew and retained its identity as a main leading hypha of the mycelial margin throughout the period of observation, mean extension rate for all main hyphae being

60·6 μ/15 minutes. Fluctuations in extension rate of a single hypha at different times were of the order of ±15 per cent. mean extension rate. Inspection of the data suggested that these fluctuations were too random to be attributable to a gross environmental drift with time or to a markedly periodic change in extension rate, e.g. in association with branch initiation. In some instances fluctuations were seen to be associated with arrestment followed by regrowth of the hyphal apex.

The conclusion that the main hyphae were potentially of unlimited growth and continued to extend in the mycelial margin with a constant mean rate over long periods of time has been abundantly supported by observations of other hyphae for periods up to 8 hours.

(*b*) *Branch initiation.* The pattern of branching of the main leading hyphae of *C. disseminatus* is essentially similar to that of other Basidiomycetes, including *M. lacrymans* (Butler, 1958). At 20° C. cell division, with the formation of a simple septum (2/3 nodes) or a clamp connexion, was restricted to the apical cell *c.* 500 μ from the hyphal apex. Cell division in the main hyphae occurred approximately every 105 minutes giving a mean internode length of 425 μ. Soon after cell division a single primary branch was almost always initiated at the apical end of the penultimate cell, close to the septum. Such primary branches were narrower than their parent hyphae and characteristically grew out at an angle of *c.* 35° to the main axis. After another cell generation time of the parent hypha about half of the primary branches had given rise to a basal secondary branch. Further branching of the parent hypha itself was rare under these conditions. In the 1,000 μ main hyphal length at the start of observations two primary branches were included and during the 3 hours of observation the system underwent between one and two branching cycles.

(*c*) *Branch development.* The twelve hyphal systems could be divided into three groups on the basis of primary branch behaviour, viz.:

(i) One primary branch was initiated at each node, all branches continuing to extend (7 systems), Fig. 2 *a*, *b*.
(ii) At least one primary branch in the observed part of the system stopped extending (3 systems), Fig. 2 *c*.
(iii) Twin primary branches were initiated simultaneously at one node (2 systems).

Considering first of all the majority group, (i), it was found that the three types of hyphae distinguished on developmental grounds, namely main, primary branch, and secondary branch hyphae, also differed markedly and consistently in extension rates (Table 1). Fig. 2 *a* and *b* illustrate the feature that within any individual system, branches of the same order but initiated at different times resembled one another closely but differed from hyphae of other orders.

Limitations of experimental procedure precluded an accurate measurement of changes in extension rate of primary branches with increasing length. Nevertheless various lines of evidence suggest that such changes, if any, were

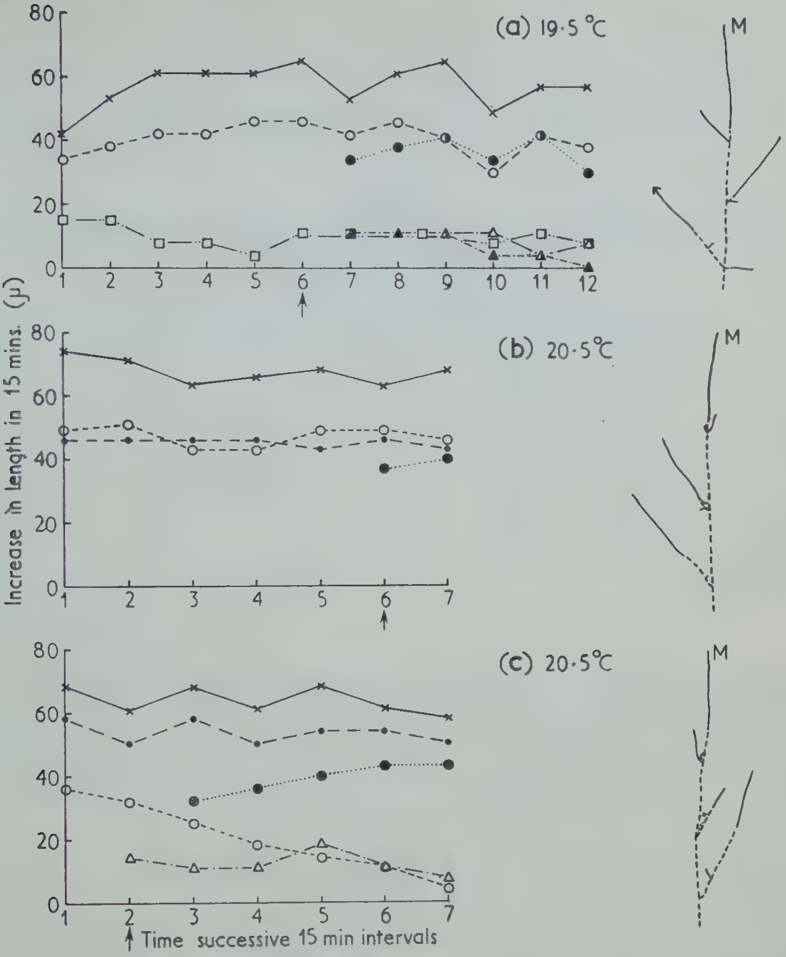

FIG. 2. Extension rates of hyphae in three individual branching systems (left) and drawings of each system (right). Nodes of the main hyphae are lettered in basipetal sequence from the tips, primary branch hyphae are denoted by arabic numerals, and secondary branch hyphae by italics and roman numerals. —x— M main hyphae; —●— Cl, --○-- Bl, ----●---- Al primary branch hyphae; —··□··— *CII*, —·△·— *CI*, ·—▲·— *BI* secondary branch hyphae. Arrows indicate the time intervals during which Al branch hyphae were initiated. In the drawings the parts of branching systems present at the start of observations are shown by dotted lines.

TABLE I

Extension Rates of Different Categories of Hyphae in Branching Systems of C. disseminatus

Hyphae	Mean ext. rate (μ/15 mins.)	Range
Main . . .	57·3	40–70
Primary branch .	38·2	26–48
Secondary branch	10·1	6–20

small, at least over the first 750 μ. Thus a comparison of the changes in extension rate of individual primary branch hyphae with those of their parent main hyphae over the same 105 minutes (mean cell generation time of main hyphae) indicated that a mean increase of 3 μ/15 min./gen. time in branch extension rate occurred (Table 2). Moreover, inspection of the mean extension

TABLE 2

Changes in Extension Rates (μ/15 mins.) of Parent and Branch Hyphae over the same 105 minutes

	Parent hypha			Branch hypha		
System	Initial ext. rate	Final ext. rate	Difference	Initial ext. rate	Final ext. rate	Difference
II	52	54	+2	38	37	−1
IV	69	66	−3	48	48	0
V	38	48	+10	24	28	+4
VI	55	59	+4	37	42	+5
VII	48	50	+2	25	31	+6
IX	59	62	+3	28	43	+15
X	73	67	−6	47	49	+2
Mean difference			+1·7			+4·4

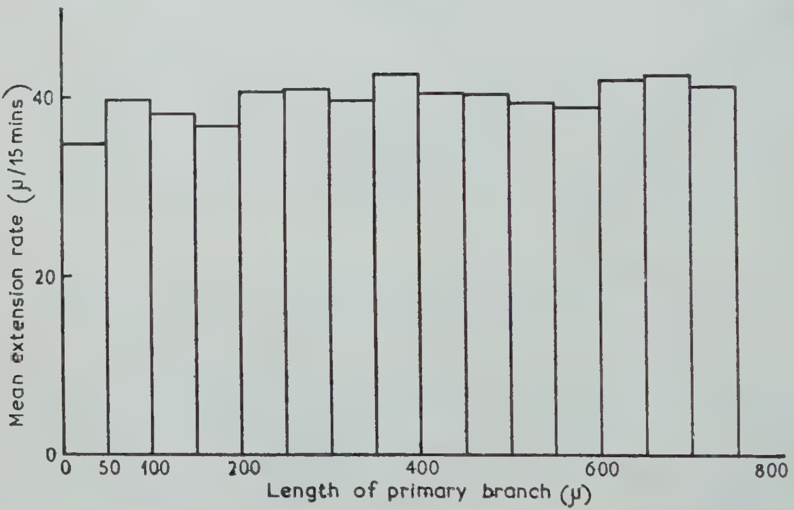

FIG. 3. Mean extension rates of primary branch hyphae in different branch length groups.

rates of all primary branches in different length groups (Fig. 3) revealed little change (*c.* 2 μ/15 min./gen. time) in extension rate over 750 μ. Where more than one primary branch was observed simultaneously in a single system the extension rates of successive branches were similar (Table 3), with once more some indication of a slight increase in extension rate with increasing length. In the early stages of primary branch growth up to *c.* 50 μ in length, as recorded by their extension during the first complete 15 minutes' interval after initiation, the extension rate did not differ significantly from the subsequent rate of the same branch. However, data for the earliest stages of branch growth were not recorded.

Thus in seven out of twelve observed systems growth was monopodial and successive new branches resembled earlier branches of the same order. However, three systems were followed in which one primary branch stopped extending (group ii, Fig. 2*c*). In all three instances this fall in extension rate of one primary branch was associated with an increase in extension rate of one or more of the other primary branches, but no corresponding change in extension rate of the main tip was evident. Although it was not clear what initiated these changes in extension rate, the data do suggest that hyphal apices within the branching system were interdependent.

TABLE 3

Comparison of Extension Rates of Sister Branches within Individual Branching Systems

	Mean ext. rate (μ/15 min.) Primary branch			Differences		
System	A	B	C	A–B	B–C	A–C
II	36·7	40·0	—	+3·3	—	—
IV	40·0	46·0	43·0	+6·0	−3·0	+3·0
V	30·3	27·7	32·7	−2·6	+5·0	+2·4
VI	33·3	38·8	—	+5·5	—	—
VII	37·0	30·8	39·3	−6·2	+8·5	+2·3
IX	42·7	42·7	51·0	0	+8·3	+8·3
X	47·6	49·2	—	+1·6	—	—
Mean difference				+1·1	+4·7	+2·0
Proportion of systems in which older branch grew faster than younger branch				4/7	3/4	4/4

2. *Analysis of Variations in Extension Rate*

In the preceding investigations there was considerable variation in extension rate not only between but also within the categories of hyphae. Moreover similar variations in hyphal diameter had been noted. Accordingly observations of the extension rate and tip diameter of forty-three individual main hyphae and their youngest primary branches were made under constant environmental conditions at 21·5° C. The growth increment of each hyphal tip during one 5-minute interval was recorded.

TABLE 4

Comparison of Growth Dimensions of Main and Primary Branch Hyphae at 21·5° C.

	Main		Primary branch		Comparison of means
	Mean	Standard deviation	Mean	Standard deviation	Probability
Extension rate (μ/15 min.)	64·6	8·00	42·2	6·16	< 0·001
Tip diameter (μ)	5·88	0·574	4·69	0·448	< 0·001

From these observations it was clear that main and primary branch hyphae formed distinct populations differing significantly in both extension rate and tip diameter (Table 4). The sample of branch hyphae varied in length from 40 to 160 μ but there were no significant changes in diameter or extension rate with increasing branch length. Considering main and branch hyphae separately, extension rate increased as tip diameter increased (Fig. 4). These two associations were statistically significant when each was tested as a linear regression. However, the extension rate of equally wide hyphae was significantly higher for main as compared with branch hyphae (probability $< 0{\cdot}001$). Thus although some of the variation in extension rate was associated with differences in hyphal diameter, this association was inadequate to explain the whole difference between main and branch hyphae.

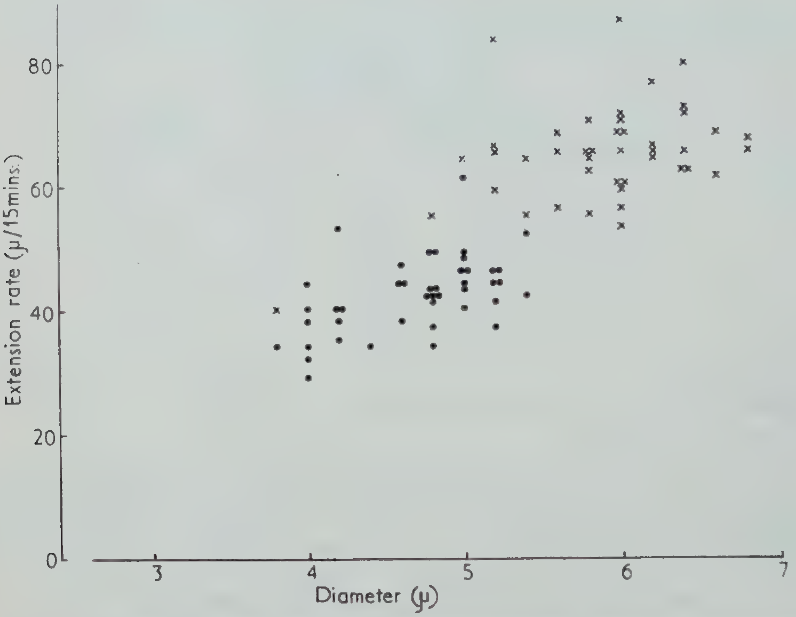

FIG. 4. Scatter diagram showing the relation between diameter and extension rate of main and primary branch hyphae. **x** main hyphae, ● primary branch hyphae.

Analysis of regression of extension rate on diameter:

For main hyphae $Y_m = 64{\cdot}607 + 5{\cdot}8179(x_m - 5{\cdot}8837)$.

Testing the significance of the regression coefficient by an analysis of variance, $t_{(41)} = 2{\cdot}946$ probability between 0·01 and 0·001.

For branch hyphae $Y_b = 42{\cdot}195 + 6{\cdot}9492(x_b - 4{\cdot}6884)$

$t_{(41)} = 3{\cdot}751$ probability $< 0{\cdot}001$.

When the significance of the difference between the two regressions for main and for branch hyphae was tested by an analysis of covariance (Mather, 1946) $t_{(83)} = 7{\cdot}015$ probability $< 0{\cdot}001$.

The total growth increment of a hypha is a function of both increase in length and diameter of the increment. Using the product *increase in length* × *diameter* as a simple measure of the total growth increment, it was found that the wider, faster-extending branch hyphae arose from the wider, faster-extending main hyphae (Fig. 5). This positive association of branch growth rate with parent growth rate was statistically significant when tested as a linear regression (probability < 0.001).

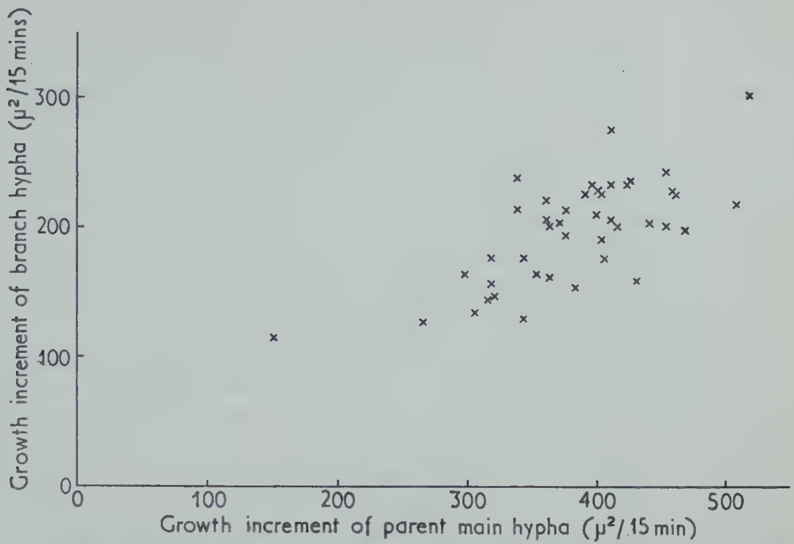

FIG. 5. Scatter diagram showing the relation between parent hyphal growth increment (increase in length in 15 min. × diameter of increment) and primary branch hyphal growth increment.

Analysis of regression of branch growth increment on parent growth increment:

$$Y = 199{\cdot}19 + 0{\cdot}43298(x - 382{\cdot}00)$$

$$t_{(41)} = 6{\cdot}31 \text{ probability} < 0{\cdot}001.$$

SUMMARY AND DISCUSSION

Apical portions of hyphal branching systems of *C. disseminatus* were observed in the margins of colonies extending at a constant rate under one set of environmental conditions. A steady state of colony extension was associated with an orderly monopodial pattern of hyphal growth. The extension rates of three groups of hyphae distinguished on developmental grounds, main, primary branch, and secondary branch hyphae, were in the proportions 100:66:18.

These observations are pertinent to the definition and understanding of the problems involved in growth of a hyphal branching system; in particular we must ask what limits the extension of a hypha and how one hypha can affect the extension of another. During growth of a hyphal apex new primary wall production occurs only at the tip (Reinhardt, 1892; Smith, 1923) whereas Zalokar (1959) has concluded that in *Neurospora crassa* protoplasm for tip extension must be supplied from a considerable length of hypha behind the tip, moving towards the tip by protoplasmic streaming. This involves both the synthesis of new protoplasm over this length of hypha and also the actual replacement of protoplasm in the older parts by vacuoles. Thus a minimum length of hypha is required for maximum extension rate of the apex. The observations of Smith (1924) support a similar conclusion for hyphae of *Botrytis cinerea*. How far this conclusion is of general application is not at present known and furthermore it remains uncertain whether the maximum extension rate on a particular medium at a particular temperature is limited by the maximum length of hypha which can contribute to tip extension, or whether some other factor or factors is important. The importance of wall development in hyphal extension has been indicated by Robertson (1959) and Corner (1947) has interpreted the regular relationship between changes in length and in diameter of basidia, cystidia and spores in terms of orderly changes in the relative rates of wall deposition and wall fixation.

It is of interest that hyphal extension rate was positively associated with hyphal diameter in *C. disseminatus*. This relationship was demonstrated amongst the variation within a population under constant environmental conditions. How far and in what way this relationship is causal requires further investigation but it does imply a common factor at some level, affecting both properties. Moreover since main hyphae and young branch hyphae (40–160 μ long) separately showed a relationship of this sort it seems that the common factor applies to both categories of hyphae.

The above discussion has neglected effects of surrounding hyphae and the distribution of growth between main and branch hyphae. Although it is a general observation that in many fungi there is a direct correlation between vigorous marginal growth and scantiness of mycelium within the culture (Brown, 1923), the two features, extension rate of marginal hyphae and branch frequency, have not been causally related.

Colony extension at a constant rate and monopodial hyphal growth with leading hyphae continuing to grow in the colony margin each occur in a variety of fungi from all major groups (e.g. Cochrane, 1958; Buller, 1931) but it is not at present clear how close is the association of these two features. Chevaugeon (1959 *a*, *b*) has contrasted the monopodial growth form and linear extension rate of wild type *Ascobolus immersus* with the sympodial growth on many media of a mutant in which the colony margin undergoes successive periods of high and very low or zero extension rate. Periodic changes in hyphal density, and thus of branch development, without any detectable changes in colony extension rate have been reported in a mutant of *N. crassa* (Pittendrigh, Bruce, Rosensweig, and Rubin, 1960). Hyphae of *C. disseminatus* were observed under conditions in which growth was ultimately being restricted by the nutrient supply, since the addition of a dilute nutrient solution to the medium increased the rate of linear extension. Here the association of linear colony extension with monopodial hyphal growth implied that the rate of extension of main hyphae remained constant and was limited by some factor which remained constant throughout the linear extension phase, when the total amount of mycelium was expanding and new branches were continually developing.

Within the individual branching system the comparatively few observations made on *C. disseminatus* provided no evidence of changes in main hyphal extension rate in association with the initiation or development of a new branch. Although Smith (1924) demonstrated that during very early growth of *B. cinerea* the rate of extension of main and branch hyphae together was proportional to the total hyphal length, his observations showed that the time at which the main apex reached its maximum extension rate (*c.* 50 hours after sowing the spores) could not be related directly to branch development, the first branch being initiated *c.* 10 hours after sowing.

Turning to consider the behaviour of branch hyphae, the data on hyphal diameters and extension rates revealed a difference between main hyphae and primary branch hyphae, the latter having a significantly lower extension rate than equally wide main hyphae. The conclusion that there was some difference in the environment of main and branch hyphae could be explained in terms of either an external factor, e.g. effects of a diffusible substrate or metabolic product, or an internal factor, e.g. nutrient competition. This environmental factor may also be significant in explaining the different extension rates of primary and secondary branch hyphae, since under the conditions of observation the position and time of initiation of each type of branch were very consistent and were markedly different from those features of the other branch type. The observation that, in the three systems where one primary branch ceased growing, adjacent primary branches increased in extension rate although the main tip was apparently unaffected suggested an interaction involving internal competition between branches.

Branch initiation in *C. disseminatus* is similar to that in other Basidiomycetes (Butler, 1958) and is strikingly regular compared with that in other groups of fungi with coenocytic hyphae and less regular cross-wall formation. Thus although branches grow more slowly than their parent hyphae in fungi from these other groups, e.g. *N. crassa* (Zalokar, 1959, Figs. 1–5), *B. cinerea* (Smith, 1924), *Chaetomium globosum* (Plomley, 1959), and *Mucor hiemalis* (personal observation) it is not clear how far the consistent branch behaviour in *C. disseminatus* is of general occurrence.

The observation that the majority of primary branches changed little in extension rate during early growth from 50 to 750 μ in length was unexpected. It was clear that subsequently some branches must increase considerably in extension rate in order to maintain the marginal density in an expanding circular colony. Further, Smith (1924) reported that branches increased in extension rate with increasing length in *B. cinerea.* However, Smith gave numerical data for only two branches and these showed little or no change in extension rate over the lengths recorded (up to 550 and 800 μ).

Other evidence that internal interactions between hyphae in a branching system occur is provided by the positive association of total branch growth increment with total parent growth increment. Without a knowledge of the relationship between diameter and extension rate it is not clear whether this could be explained in terms of an association of branch diameter at initiation with parent diameter.

It is evident that under these conditions neither the parts of individual branching systems nor the separate systems can be considered in isolation. A solution of the problems indicated by this analysis is essentially quantitative since the relative magnitudes of the characters vary with the environment and are thus susceptible to experimental investigation. The characters which have been measured (increase in length and tip diameter) are merely convenient ones and may well not directly correspond to more fundamental processes.

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Changes in the Amount and Distribution of Increment Induced by Contrasting Watering, Nitrogen, and Environmental Régimes

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With one Plate and one Figure in the Text

ABSTRACT

Young apple-trees were grown for one season under various conditions; the increments in dry weight and the proportions of these increments distributed to leaves, stem, and roots were determined. Considerable differences in both increment and distribution were induced by watering, nitrogen, and a greenhouse environment. Much of the change in distribution could be attributed to the treatments without any effect of the size of the increment, but in the cases of new stem under all treatments in the greenhouse or in the open, and of root in the greenhouse, changes in proportion were not definitely independent of change in increment.

These environmental treatments, in their effect of changing the pattern of distribution, are to be contrasted with treatments such as pruning and shoot number which only alter the proportion of old stem to new stem, and then only according to the degree of pruning. It is concluded that a distinction, useful in analysing the growth of woody perennials, may be made between these two groups of treatments. One group affects the physiology of the plant; the other acts chiefly by switching the 'form' of the developing plant without affecting the general physiology.

INTRODUCTION

THE growth analysis of fruit trees sometimes involves comparisons between trees which, although of the same age, have, as a result of rootstock or pruning differences, grown at different rates and may differ considerably in size. Such trees differ in number and length and thickness of shoots, numbers of long shoots and spurs, and in other less apparent ways. A series of experiments on how these 'morphological' differences themselves affect the subsequent increment in weight and its distribution to leaves, stem, and root led to the conclusion that the pattern of distribution was stable (Maggs, 1958–60). But size differences may also be due to soil and site differences; information on the effects of such site factors on the distribution of increment in woody species is scanty. The most widespread factors are probably nitrogen and soil moisture; Mason (1958) and Ruck and Bolas (1956) have reported that these may affect the total increment and its distribution. Further information was required on these factors and their interaction with climate.

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MATERIALS AND METHODS

The treatments chosen for this experiment were therefore nitrogen status, watering régime, and the environmental differences between an unheated greenhouse and the open air. Nitrogen and water were given at two levels, and the four combinations were grown both in the greenhouse and in the open air. Rooted shoots of the apple-rootstock M.XVI were carefully selected, pruned to half their length, and on February 3, 1959 potted in 9-inch (23-cm.) clay pots. There were 8 replicates of each treatment, making 64 plants in all. Samples were taken for estimating initial dry weight. The treatments were applied just after bud-break. All pots were covered with black polythene film to prevent the entry of rain. On May 9, when the upper shoots were about 10 cm. long, the plants were disbudded to one shoot per plant. All trees were given a general nutrient solution (nitrogen-free) in July and August. They were harvested on September 30.

TABLE 1

Water Used per Plant (litres) July 14–September 29

	In open (*F*)		In greenhouse (*f*)	
	N	*n*	*N*	*n*
W	13·0	13·0	15·0	13·5
w	7·5	7·6	7·3	7·6

Watering régime. The plants under the high water régime (*W*) were watered to field capacity whenever the surface soil became dry—every day or every other day; those under the low water régime (*w*) were watered whenever the shoot-tip was drooping at 9.00 hr.—about every fourth day. Two plants in each treatment were weighed before watering and after surplus water had drained off. The total water used per plant is summarized in Table 1.

Nitrogen. The trees were potted in sterilized compost (J.I.) from which in the case of the low nitrogen treatments (*n*) the supplement of hoof and horn meal had been omitted. 2 g. ammonium nitrate were added to each of the high nitrogen (*N*) pots every fortnight Only in August and September were the leaves of the low nitrogen plants obviously paler; their dried leaves averaged 3·6 per cent. nitrogen against 4·1 per cent. in the *N* leaves.

Greenhouse. The greenhouse was an unheated dutch-light structure, and the plants were stood in four rows in the centre of the cinder floor. Gross overheating in summer was avoided by painting 'Summer Cloud' on the overhead glass, by keeping the doors and ventilators open most of the time, and by frequently damping down. In the open, the plants were not sunk in the ground because water might be taken in through the walls of the pots, but the pots were surrounded by straw to reduce water loss from their surfaces.

RESULTS

Plate 1 is a photograph of the trees at harvest. The largest and fifth largest tree of the eight trees in each treatment is shown to give an idea of the variation as well as mean size. It is apparent that the *W* treatments made more growth than the *w*, but that *N* and *n* treatments showed little difference. Plants from the greenhouse were a little taller than those grown in the open. Leaf areas on four of the replicates were measured on June 6; these showed that differences had already begun to develop, and subsequent measurements showed that in each environment the pairs of treatments receiving similar water régimes (*FNW* and *FnW*, *FNw* and *Fnw*, &c.) increased their leaf surfaces at similar rates until August when the *N* treatments grew a little faster. The average leaf area per plant is given in Table 2, and the increase in dry weight from planting in Table 3.

TABLE 2

Total Leaf Area per Plant on September 15 (sq. cm.)

	In open (*F*)		In greenhouse (*f*)	
	N	*n*	*N*	*n*
W	1542	1293	2331	1941
w	990	657	1149	1314

TABLE 3

Increase in Dry Weight from February 4 to September 30 (g.)

	In open (*F*)		In greenhouse (*f*)	
	N	*n*	*N*	*n*
W	95·88	94·73	83·54	82·39
w	43·37	46·01	35·30	41·02
	s.e. 4·13		2·52	

The dry weights confirm the opinion gained visually that reduced water-supply severely checked growth; it is also apparent that the plants grown in the open, though shorter were also heavier than those from the greenhouse. In the greenhouse, extra nitrogen decreased the weight of the low water plants, but elsewhere it was without significant effect on increment.

For each plant the total increment during the season was divided into leaf, new stem, old stem increment, and root, and the weights of these parts were calculated as a fraction of the total increment. Old stem increment was the difference between old stem weight and estimated planting dry weight. These fractions (as percentages) are summarized in Table 4. In general, the biggest differences appeared between the greenhouse and field environments. In the greenhouse there was relatively more leaf and new stem, a little less old stem, and much less root. Within each environment, nitrogen and watering affected different parts of the plant. Nitrogen notably increased the percentage of leaf and depressed correspondingly the other parts, although in the field this later effect did not quite reach significance; in particular root percentage tended to be depressed by nitrogen in the presence of adequate water. Watering notably increased percentage of new stem and depressed old stem increment and root, again with different emphasis in field and greenhouse. There were also significant differences in the water content of leaves and new stem, adequate water increasing the water percentage of the leaf by about 2 per cent., and nitrogen that of the new stem by about 2·5 per cent., thus reversing their effects on dry-weight distribution.

TABLE 4

Percentage Distribution of Increment

						Significance		
	WN	*Wn*	*wN*	*Wn*	Sig. diff. for sub-trt. means	*W*	*N*	*W×N*
In Field								
Leaf	25·9	20·7	27·4	21·2	*2·2*	*N.S.*	*0·001*	*N.S.*
New stem	26·9	27·2	21·4	22·9	*2·1*	*0·001*	*N.S.*	*N.S.*
Old stem increment	26·2	26·6	26·8	29·9	*2·6*	*0·05*	*N.S.*	*N.S.*
Root	21·0	25·5	24·4	26·0	*4·7*	*N.S.*	*N.S.*	*N.S.*
Total stem	53·1	53·8	48·2	52·8	*3·7*	*0·05*	*0·05*	*N.S.*
In Greenhouse								
Leaf	37·1	28·6	37·6	30·4	*3·4*	*N.S.*	*0·001*	*N.S.*
New stem	31·3	34·0	26·4	29·1	*1·3*	*0·001*	*0·001*	*N.S.*
Old stem increment	20·9	24·0	21·3	25·2	*2·3*	*N.S.*	*0·001*	*N.S.*
Root	10·7	13·4	14·7	15·3	*2·5*	*0·01*	*N.S.*	*N.S.*
Total stem	52·2	58·0	47·7	54·3	*2·7*	*0·001*	*0·001*	*N.S.*

DISCUSSION

Treatment effects. The main effects of the treatments are in general agreement with previous work. The distribution of increment calculated from Mason's data (1958) on a similar watering régime showed that drought reduced the relative amount of new stem by 10 per cent., the main corresponding increase being in old stem and root. Harris (1914) obtained similar results with other genera. Batjer and Degman (1940) and Ruck and Bolas (1956) found nitrogen to increase significantly dry weight of young apple-trees, and its lack of effect in this experiment may be attributed partly to the release of nitrogen from a sterilized soil and partly to the adequacy of nitrogenous reserves in the old stem (Harley *et al.*, 1949). However, it has been the experience at this station that in sand culture nitrogen-deficiency symptoms occur well before the end of a single growing season (Delap, personal communication). The increase in leaf percentage is a general effect of nitrogen, although Ruck and Bolas (loc. cit.), Table III, found little such effect; they did find, however, a considerable increase in stem percentage and a decrease in root. Their data for 200 p.p.m. nitrogen is not conclusive as these plants were grown for two seasons, and leaf percentage decreases considerably in the second year (Maggs, 1960*a*).

In comparing the treatments simulating climatic differences—field and greenhouse—some of the differences were similar to those resulting from shading (Maggs, 1960*b*), viz. slightly reduced increment, and increased leaf percentage and decreased root percentage. In the present case, however, the changes in leaf and root percentage did not balance out and there were significant differences in total stem percentage (Table 4). It may be noted that *Wn* produced a much higher percentage of total stem in the greenhouse than in the field. It is likely that the different temperature régimes also contributed to the differences. Nightingale (1935) has described the more rapid course of root differentiation at higher temperatures, which would lead to the production of roots with a higher dry-weight/fresh-weight ratio; at the same time, the build-up in the soil of respiratory CO_2 would be more rapid, and this in turn would depress root growth (Boynton, 1939; Boynton and Compton, 1943). Hancock and Barlow (1958) described a positive linear relation between shoot growth and the accumulated temperature averaged over the preceding 5 days, while Gregory (1926) found evidence for a negative correlation between net assimilation rate and night temperature. While it is clear that climate can induce differences in distribution, it would be rash to designate any single factor as preponderant in evoking the observed differences.

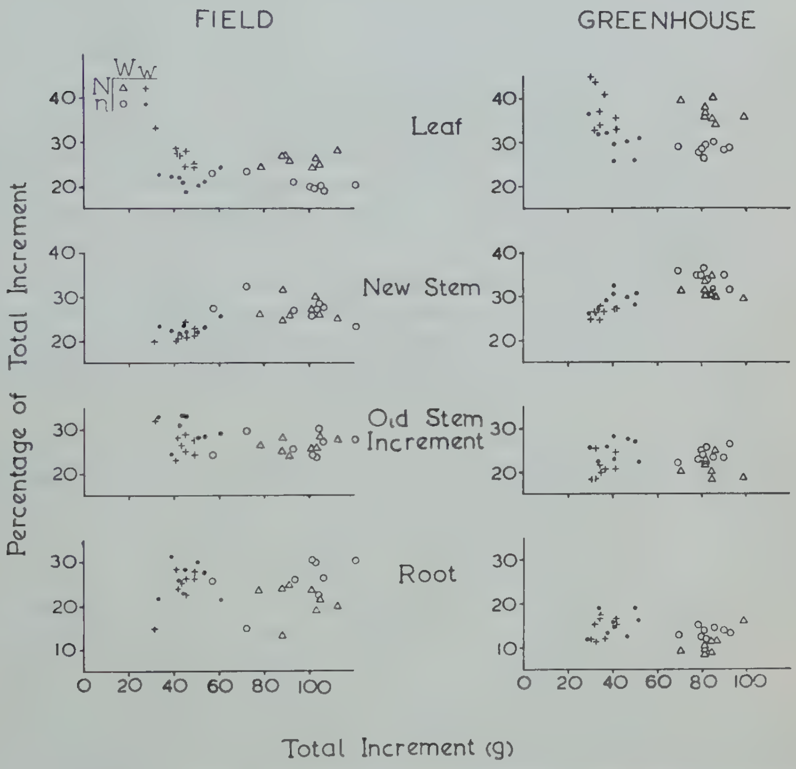

FIG. 1. The relation between total increment and percentage of each part in the increment. The points for each part do not lie on a single curve, showing that the changed distribution was not due to differences in the amount of increment. Possible exceptions are new stem in field and greenhouse and root in greenhouse.

Relations between percentage part and increment. The experimental treatments produced differences both in the size of the increment and in its distribution, and the first point to consider is how far the one is a cause or an effect of the other. Thus it might be argued that a greater increment was due to a higher percentage of leaf, or again that a higher proportion of stem was a result of a greater increment for the reason that as a tree grew faster more of the increment was deposited in the branches near the site of production. If amount and distribution of increment were thus interrelated, a plot of these two variables for all trees should tend to lie on a smooth curve, or at least for trees under a common treatment, e.g. *W* (*Wn* and *WN*) or *n* (*Wn* and *wn*), &c. These plots are shown in Fig. 1. It is clear from the figures that in most cases treatment differences in distribution cannot wholly be explained in terms of changing increments. A more detailed analysis of the mathematical correlations between percentage part and increment showed that only in the cases of stem-within-greenhouse and within-field and of root-within-greenhouse could the change in one account for a significant part of the change in the other. Even conceding these relations (in agreement with Maggs, 1960*a*), it is still clear that much of the change in form resulting from the treatments cannot be accounted for by a constant relation between percentage part and total increment and therefore the treatments themselves have altered the pattern of distribution of increment.

Two groups of treatments. Maggs has reported on a series of experiments on young trees and if all the results are considered, the treatments may be divided into two groups—those which do and those which do not seriously disturb the pattern of growth. These experiments are briefly summarized below.

Treatment	*Results*
Time and variety (Maggs, 1958, recalculated from the data)	After allowing for different dates of bud-break, the variety M.II differed from M.XVI in that less of the increment was utilized as root, and more as old stem thickening.
Pruning (Maggs, 1959)	Leaf, stem, and root tissues comprised constant fractions of the total increment. The relative amounts of new stem and old stem thickening could be linearly related to the degree of pruning.
Number of new shoots, variety (Maggs, 1960*a*)	Despite big differences in total increment, the distribution of that increment was scarcely affected by the number of shoots that the plant bore, both in the planting year and in the subsequent year. Varietal effects were small. In the first year—the establishment year—leaf percentage was higher and old stem increment lower than in the second year.
Shade (Maggs, 1960*b*)	Although shading had consistent effects on the seasonal pattern of distribution of increment, such effects were small compared with the seasonal variation. Shading increased the relative amount of leaf and decreased root, while the stem fractions were little changed by treatment.
Nitrogen, water, 'environment' (present paper)	The pattern of distribution was altered by all the treatments, although in the case of the stem regions, the effect of nitrogen and water might have been due to an effect on the amount of increment.

Thus the group of treatments without effect on the pattern of distribution includes pruning (1959) and shoot number (1960*a*); and the group of treatments seriously modifying the distribution includes variety (1958, 1960*a*), water (Mason, 1958, this paper), and nutrition (Ruck and Bolas, 1956; Delap and Ford, 1958, this paper). Each set of treatments has a common character to which this effect on increment may be ascribed. In the first set the treatments are switch-like in action so that the form of the growing tree is changed from the instant that the treatments have been applied; the final differences result directly from these initial differences. The second group alter the physiology of the tree or treated part, and are usually operative for the whole or for a large part of the subsequent growing period; the final differences are the cumulative expression of the continuously altered physiology.

The two groups cannot be absolutely distinguished, since differences originally only in form may entail changes in physiology—as when a potted plant with two shoots becomes droughted earlier than one with a single shoot—and vice versa, differences in physiology will lead to differences due to the subsequent changes in form—as when a heavily cropping tree snaps an inadequately thickened bough. Nevertheless, usually treatments on the form of the tree will not be followed by the abrupt changes in the *pattern* of growth that may result from physiological treatments.

Shading provides an interesting border-line example (Maggs, 1960*b*). The main effect of shading was a proportional reduction in net assimilation rate leading to a disproportional reduction in absolute increment, while the pattern of distribution over the season remained roughly the same whether the increment was 10 g. or 100 g. All the same, physiological changes entailing changes in distribution did occur, principally expressed in the increased leaf and decreased root proportion together with a great increase in the lamina dispersion, and these had the result that the increment was about twice that to be expected in the absence of modification.

The analysis of the rootstock effect in apple-trees, initiated by R. C. Knight and M. C. Vyvyan, requires the separation of both groups of factors. One of the effects of grafting a scion variety on to different rootstocks is that the various combinations grow at different rates; within a few years, tenfold differences in weight may have developed. In part these differences are due directly to the physiological differences between the rootstocks, but partly they are due to the differences in form initiated with the outgrowth of the scion, such as reduced shoot number and lower leaf proportion. Thus Vyvyan (1955) has shown that such contrasting varieties as M.XVI and M.IX only differ in relative growth-rate for little more than the first year after grafting, which suggests that thereafter 'form' factors predominate in perpetuating the size differences. With the onset of cropping, a further end-point for synthesis is introduced; the fraction of the total increment formed into crop must perforce vary considerably with environmental conditions; nevertheless, Fisher (1934), Hatton (1935), and Ryugo and Davis (1959) have demonstrated a marked effect of variety on the partition between crop and other growth. How far this may be due to the consequences of simple competition for metabolites, and how far the crop itself has induced changes in the distribution does not yet appear to have been determined.

SUMMARY

1. Selected rooted cuttings of the apple rootstock M.XVI were grown for a season under two levels of watering and nitrogen either inside a greenhouse or in the open.

2. The higher level of watering approximately doubled the increment; nitrogen had practically no effect; the greenhouse environment slightly reduced it (Table 3).

3. When the increment was subdivided into leaf, new stem, old stem increase, and root, the percentages of the increment that these comprised were altered by all treatments. Water increased the percentage of new stem, nitrogen increased the percentage of leaf, and the greenhouse environment increased the percentage of leaf and decreased that of root (Table 4).

4. In the cases of new stem within each environment and of root within greenhouse, a significant part of the change in percentage part could be associated with change in increment. For the rest, it appeared that treatments altered the percentage of the part directly.

5. A distinction is drawn between treatments which modify the physiology of the tree for most of the growing season and those which switch the direction of early development. The former usually alter the overall distribution of increment to leaves, stem, and root, the latter do not.

ACKNOWLEDGEMENT

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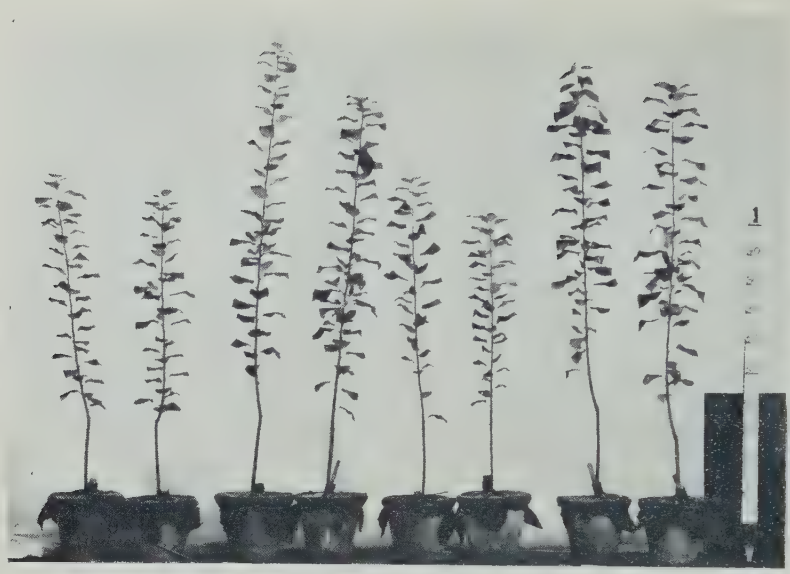
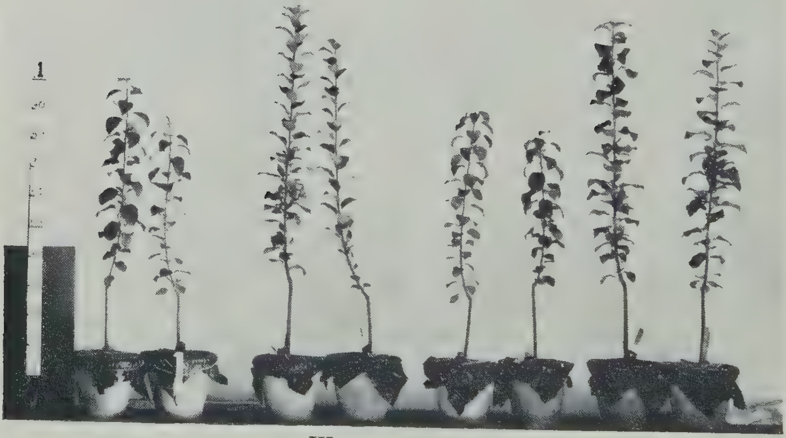


Grown in greenhouse



Grown in field

D. H. MAGGS

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EXPLANATION TO PLATE

Largest and fifth largest of the eight trees in each treatment. *W*—high water régime, *N*—high nitrogen régime.

Pit-field Distribution in Relation to Cell Growth in Dwarf Peas, as Affected by Gibberellic Acid

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With two Figures in the Text

ABSTRACT

Data are given relating cell length and pit-field frequency to internode length, as influenced by the application of gibberellic acid. Both cell extension and cell division are affected, the latter especially in internodes developing later after treatment. Where enhanced cell extension only occurs, existing pit-fields merely become spaced out. Where cell division is involved, this is accompanied by an increase in pit-field numbers so that daughter cells of the same type tend to have a characteristic number. This is in accordance with earlier observations on other plants and confirms the suggestion made previously of the existence of rather different patterns of cell-wall extension in dividing, as distinct from merely enlarging, cells.

INTRODUCTION

THE relevance of pit-field distribution to problems of cell-wall growth was pointed out in 1955 by Wardrop, who showed that the number of pit-fields in parenchyma cells of *Avena* coleoptiles remains approximately constant during the growth in length of the coleoptiles from about 10 to 60 mm. In this final phase of coleoptile extension cell division has ceased, the cells merely elongate, and during this elongation the pit-fields retain their more or less uniform distribution over the contact faces of the cell-wall. Thus the growth of the latter may be visualized as intussusceptive in character, giving rise to a general spacing out of pit-fields already existing in the young cell. At about that time I had begun some rather similar observations on pit-field distribution in the cortical parenchyma of *Elodea* and subsequently these were extended to the corresponding tissue in *Hippuris* (Wilson, 1957, 1958). These two plants provide rather favourable material for observations of this kind in that, *inter alia*, there is in each, behind a growing apex, a long series of internodes of gradually increasing length, so that the elongation of the tissue as a whole may be conveniently quantitatively referred to the transverse division and elongation of the cells of which it is made up. In *Elodea* cortex, in internodes longer than about 0·5 mm., cell division may in this way be inferred to have ceased, and the behaviour of the pit-fields tends to that of those of *Avena* coleoptiles described by Wardrop. Growth in these longer internodes involves cell extension without cell division, and the number of pit-fields in

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one cell, and similarly in the tissue as a whole, remains approximately constant. In younger internodes, however, the situation is more complex, in that the number of pit-fields in a 'unit' of cell-wall increases as that 'unit' grows: the pit-fields do not merely become spaced out, they become divided, apparently by intussusception of new wall material within themselves. In these internodes, moreover, the growth of the tissue as a whole involves transverse cell division in the cortical parenchyma, so that the increase in pit-field numbers is correlated with this. This seems, on general grounds, to be an understandable correlation, since the continuance of transverse division in cells whose longitudinal growth involved merely a spacing out of existing pit-fields would result ultimately in the production of cell progeny lacking them altogether. In *Hippuris* cortex, which is similar in general form to that of *Elodea*, cell division continues during growth and correspondingly the pit-field numbers go on increasing, so that the final appearances of the cell walls in mature internodes in the two cases are quite different. This may be seen by comparison of Fig. 4 of Wilson (1957) and Fig. 5 of Wilson (1958).

If this correlation between the types of growth and pit-field distributions is a real one it implies a different pattern of cell-wall growth in cells which are dividing, as compared with that of their later progeny in which cell division has ceased, and in which extension only is going on. This is a complicating factor in any general theory of the cell-wall-growth process. In the observations to be described, further evidence on this matter is adduced from tissues in which growth has been experimentally varied by the application of gibberellic acid. Although the stimulation of growth so produced is, to a large extent, by way of cell enlargement (Brian, 1959), this may not be entirely so, as Feucht (1958) and Greulach and Haesloop (1958) have shown. Both cell division and cell enlargement may be affected, and additional evidence on the distribution of pit-fields in relation to both these processes has been obtained.

TECHNIQUE

The plant material and gibberellic acid treatment applied to it were essentially those of Brian and Hemming (1955). Dwarf peas (variety *Meteor*) were grown in John Innes compost in the greenhouse and were stimulated into elongated growth by a single application of gibberellic acid in alcoholic solution to the first fully differentiated leaf. Since the maximum effect was aimed at, for which it seems from this work that the amount to be applied is not very critical, application was made from a finely drawn capillary tube calibrated so that an estimated 2·5–3 mm.^{3} of solution, containing about 6–8 μg. of gibberellic acid, could be delivered. A preliminary trial of this kind produced a reasonably uniform stimulation of growth which closely paralleled that described by Brian and Hemming. Subsequently, two separate batches of experimental plants were grown, on separate occasions. From these, smaller groups were selected for uniformity of development, and individuals were paired for use as 'treated' and 'control' plants. The maximum internode lengths attained were noted. Samples were taken from various internodes, embedded and sectioned, and estimates were made of cell length and pit-field frequency in certain tissues. These were based on fifty to a hundred cells of each type drawn from two samples of stem in each internode studied. Pit-field frequencies were estimated either by counting the number in a standard area of wall (with the aid of a graticule eyepiece) or else by reference to the total number appearing in a radial face of a cell (see below). They were made visible by the use of a polarizing microscope fitted with an elliptic compensator, and used with a high-pressure mercury-vapour lamp. The compensator was adjusted so that its birefringence was additive to that of the specimen: over a small range of adjustment this procedure increases the apparent brightness of the birefringent material in the cell wall proportionately more than that of the background, so that the contrast is increased very appreciably.

RESULTS

In the first group of plants the first fully differentiated leaf was fairly well expanded at the time of treatment, while later internodes were still short and enwrapped in the stipules of this leaf and later leaves. The mean final internode lengths attained for the first four internodes above the treated leaf are given in Table 1 (data to the nearest 0·5 mm.). The internodes below the treated leaf were at, or very near, the end of their normal extension phase and did not respond to the treatment.

TABLE 1

Mean Final Lengths of Internodes (mm.) (First Group)

No. of internode above treated leaf	Length controls	Length treated
1	13±1·5	23±2
2	20·5±1·5	67±2·5
3	28±1·5	78·5±4·5
4	31±1·5	61·5±5

In these plants inspection indicated that although the enhanced extension growth of the treated plants could in large degree be referred to increased cell enlargement, especially in the pith, this was clearly not wholly true. The sub-epidermal chlorenchyma of the outer cortex (in the regions between the vascular bundles) were apparently much less affected in size by the treatment: they had clearly increased in numbers in the treated plants. Attention was therefore directed to a more detailed examination of the unspecialized parenchyma of the pith and inner cortex, and of the sub-epidermal chlorenchyma. However, the pith tends to break down in the higher internodes, so that the data referring to it could be obtained only for the first internode above the treated leaf: the residual outer pith of the later internodes grades rapidly into bundle-sheath tissue of longer cells and is difficult to sample for measurement on this account. Even the cortical parenchyma is less satisfactory than a central pith from this point of view, since it is more variable and less extensive, being virtually completely interrupted opposite the vascular bundles by strands of fibres.

The data referring to mean cell length and pit-field frequency in the four internodes above the first fully differentiated leaf in treated and control plants are given in Table 2. These are based on detailed observations on five plants of each drawn from the larger batch grown. In parenchyma cells, where on account of their size it is rare for whole cell faces to appear clearly in one section, the pit-field frequencies refer to the number appearing in a smaller arbitrary standard unit area of the wall. The pit-fields in these cells are fairly uniformly distributed, and sampling of this kind is appropriate. In the chlorenchyma cells, however, pit-field distribution is markedly non-uniform, since the cells are slightly lobed and correspondingly there are intercellular spaces between them. Here, therefore, as the cells are much smaller, and are more frequently seen in their entirety, the total number of pit-fields in a radial face was recorded. The table has not been burdened with the standard errors of these means; these fall between about 3 per cent. and 8 per cent. of the means themselves. There is some difficulty in this matter, especially in that the counts of pit-field numbers involve an element of subjective judgement (e.g. at what stage does one pit-field become two) which the observer can only try to reduce to a minimum by the use of standard conditions of illumination and magnification and a conscious guard against unconscious bias. Some of the observations have been repeated after long intervals, and have been found to show satisfactory agreement, so that I consider that reasonable accuracy has been maintained. Another observer might return different absolute figures, but, I should expect, closely similar relative results.

The mean ratios (treated/control) of the data of Table 2 (*a*) are given in Table 2 (*b*). Since the internode lengths given are the distances between corresponding points at successive nodes, a correction has been applied in calculating their mean ratios, as representing the approximately constant length of the leaf-base insertion. From Table 2 (*b*) clear indications emerge of the types of growth taking place.

In the first internode the ratios of cell lengths are close to those of internode lengths, so that in all three tissues considered the enhanced growth is simply a matter of enhanced cell extension. The inverse ratios of pit-field frequencies in the parenchyma cells are close to the ratios of cell lengths so that by simple proportion the number of pit-fields in these cells remains approximately constant. In the chlorenchyma cells this is evident directly from the counts of the total number in a cell face. Thus here, as in older coleoptiles and *Elodea* cortex, cell enlargement involves a 'spacing out' of pit-fields present in the wall. This is illustrated in representative pith cells in Fig. 1.

In the higher internodes, which were progressively younger at the time of treatment, the situation is rather different. The ratios of cell lengths fall below those of internode lengths, while the inverse ratios of pit-field

TABLE 2

(a) Mean Internode Length, Cell Length, and Pit-field Frequency in Four Internodes above the First Fully Differentiated Leaf

	Pith parenchyma		Cortex parenchyma		Sub-epidermal chlorenchyma	
Internode length (mm.)	Cell length (μ)	P-f. freq.	Cell length (μ)	P-f. freq.	Cell length (μ)	P-f./cell
1st internode						
Control						
13	234	23·8	162	21·6	55	17·8
15	229	23·1	175	21·8	47	19·8
14	242	22·0	190	21·4	54	20·1
14	213	24·2	178	25·2	53	21·9
10	220	23·9	161	19·7	55	21·5
Treated						
25	538	8·2	307	11·9	105	21·8
21	466	8·9	356	12·1	84	21·1
24	366	11·8	325	14·6	99	23·2
29	506	8·5	315	9·1	108	21·1
28	495	9·0	327	11·2	116	24·9
2nd internode						
Control						
22	—	—	181	23·3	49	18·2
25	—	—	184	21·6	53	14·4
14	—	—	170	20·1	51	18·1
21	—	—	168	28·5	45	19·2
17	—	—	169	19·0	50	18·9
Treated						
69	—	—	487	6·7	74	17·9
66	—	—	441	7·2	73	14·8
65	—	—	347	8·8	76	18·1
76	—	—	511	7·8	84	21·2
57	—	—	504	6·8	97	21·1
3rd internode						
Control						
29	—	—	133	23·4	33	17·0
33	—	—	123	22·9	37	16·3
22	—	—	163	19·6	46	15·7
27	—	—	140	20·0	43	18·0
29	—	—	128	19·5	50	17·4
Treated						
95	—	—	228	11·0	51	16·0
72	—	—	264	10·8	54	15·5
58	—	—	177	11·3	50	14·2
85	—	—	331	7·7	63	17·0
72	—	—	276	9·5	59	15·8
4th internode						
Control						
33	—	—	107	25·2	38	16·5
32	—	—	111	26·2	38	16·1
26	—	—	119	22·4	43	16·8
30	—	—	112	22·2	40	16·0
29	—	—	107	23·9	35	15·6
Treated						
74	—	—	200	15·4	55	15·8
60	—	—	182	17·3	51	17·4
40	—	—	142	17·3	43	17·5
69	—	—	197	12·9	58	15·8
53	—	—	167	16·0	47	16·5

(b) Mean Ratios (treated/control)

		Pith cells		Cortex cells		Sub-epidermal chlorenchyma	
Internode no.	Internode length	Length	1/P-f. freq.	Length	1/P-f. freq.	Length	P-f./cell
1	2·04	2·08	1·89	1·88	1·86	1·91	1·11
2	3·56	—	—	2·63	3·02	1·62	1·05
3	2·83	—	—	1·86	2·10	1·31	0·93
4	2·03	—	—	1·60	1·52	1·31	1·03

frequencies in parenchyma are closer to those of cell length rather than to those of internode length, and the number of pit-fields in chlorenchyma cells remains unchanged by treatment. Some additional cell division has therefore taken place, and correspondingly, the pit-field numbers have increased to maintain the number per cell at a characteristic level. This is especially so in the chlorenchyma, of which representative cells are illustrated in Fig. 2.

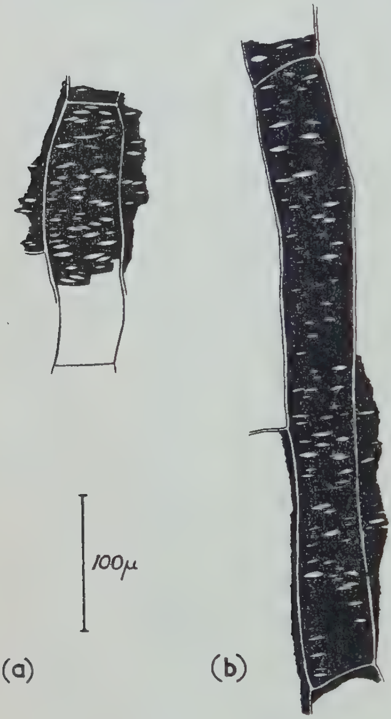

FIG. 1. Representative pith cells (*a*) control, (*b*) gibberellic acid treated, from the first internode above the first fully differentiated leaf. Enhanced growth does not involve additional cell division; the pit-fields become spaced out. Cf. Table 2.

In the second group of plants essentially the same relationships appeared, though with variations arising from the somewhat different stage of development at which the gibberellic acid treatment was applied. The first internode above the treated leaf was near the end of its normal extension and the second internode was also fairly well extended. The gibberellic acid was applied as before and the results in terms of mean internode lengths are given in Table 3. Although it is not evident from the mean data, it is perhaps noteworthy that the final lengths of the second internodes of treated plants were negatively correlated with their initial lengths: the earlier in the extension of the internode that the gibberellic acid was applied, the greater its ultimate effect ($r = -0{\cdot}65$, highly significant). Control plants showed no such correlation ($r = +0{\cdot}2$, not significant).

In this group of plants changes in cell length and pit-field frequency clearly occurred in the same general way as in the first group: details are given in Table 4 for the second and fifth internodes above the treated leaf. Among the second internodes of treated plants which were examined in detail, however, two sub-groups were recognized; (*a*) those of plants which had been treated early in their extension and had therefore achieved maximum final length, and (*b*) others which were slightly more advanced had thus been treated relatively later and had shown less final response.

It is clear that here again, in the second internode, where extension was well under way at the time of treatment, the enhanced growth of the tissues studied was, in both sub-groups, almost wholly referable to cell extension, and the pit-field frequencies behaved in accordance with expectation,

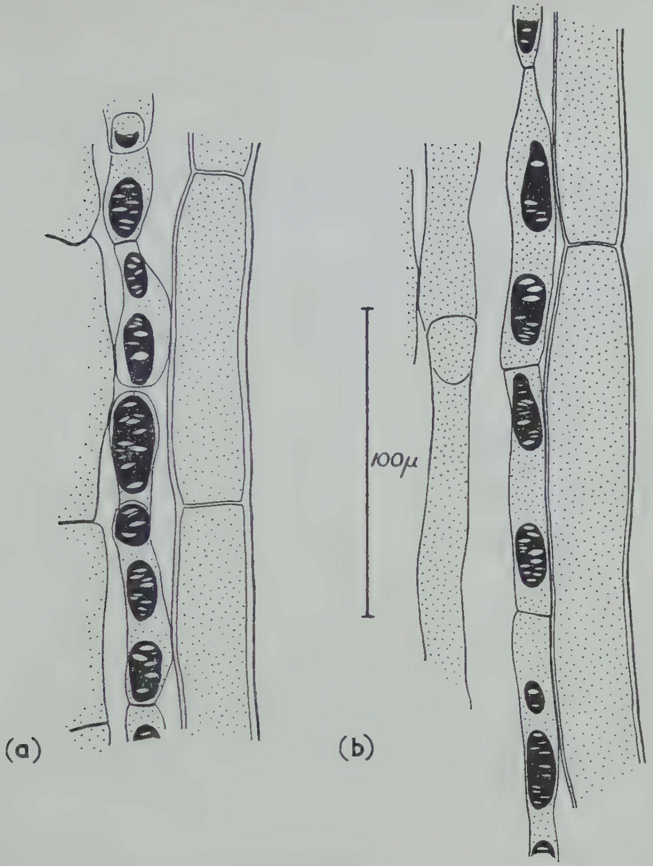

FIG. 2. Representative sub-epidermal chlorenchyma cells, (*a*) control, (*b*) gibberellic acid treated, from the second internode above the first fully differentiated leaf. Enhanced growth here involves additional cell divisions. Nevertheless, the characteristic number of pit-fields in a cell face is maintained. Cf. Table 2.

TABLE 3

Mean Lengths of Internodes (mm.) (Second Group)

No. of internode above treated leaf	Controls		Treated	
	Initial	Final	Initial	Final
1	12±0·5	13±0·5	11·5±0·5	14±1
2	9·5±0·5	18·5±0·5	9·5±0·5	39±2
3	—	21±1·5	—	71·5±2·5
4	—	22·5±1	—	86·5±2·5
5	—	23·5±1·5	—	76·5±3

indicating a spacing out of pit-fields in growth. In the fifth internode, however, some additional cell division resulted from the gibberellic acid treatment and, as before, pit-field frequencies are relatable to cell length, not to internode length. Again, pit-fields have increased in numbers in the process.

TABLE 4

(*a*) *Mean Internode Length, Cell Length, and Pit-field Frequency in the Second and Fifth Internodes above the Treated Leaf* (*Second Group*)

	Cortex parenchyma		Sub-epidermal chlorenchyma	
Internode length (mm.)	Cell length (μ)	P-f. freq.	Cell length (μ)	P-f./cell
2nd internode				
Control				
19	171	19·2	48	20·4
19	155	20·8	41	20·8
18	133	25·0	43	21·5
18	159	23·3	48	20·4
Treated				
(*a*) 51	361	10·1	102	24·2
54	323	10·2	101	21·4
49	368	9·0	113	23·4
45	346	10·1	86	22·3
(*b*) 31	233	13·3	64	21·9
25	252	13·5	65	23·4
29	272	13·3	69	22·2
35	310	10·9	75	19·0
5th internode				
Control				
23	84	24·1	42	16·3
27	84	25·2	35	14·3
23	108	23·8	45	15·3
24	92	22·4	37	14·7
Treated				
72	149	12·2	43	15·0
80	183	11·3	51	12·4
90	183	10·1	56	15·3
76	160	14·4	40	14·9

(*b*) *Mean Ratios* (*treated/control*)

		Cortex parenchyma		Sub-epidermal chlorenchyma	
Internode no.	Internode length	Length	1/P-f. freq.	Length	P-f./cell
2 (*a*)	2·83	2·26	2·23	2·22	1·10
(*b*)	1·68	1·74	1·73	1·47	1·04
5	3·43	1·80	1·99	1·20	0·95

DISCUSSION AND CONCLUSIONS

In short, therefore, the data here presented extend and confirm the earlier observations on other plants. In enlarging cells the pit-fields remain constant in number, becoming spaced out as the cell grows. In dividing, as distinct from simply enlarging, cells, the pattern of intussusceptive growth of the cell wall is somewhat different and results in the multiplication of pit-fields. The effect of this is that there is a characteristic number in any cell of the same type in a given internode, though there is evidently, in these dwarf peas, a longitudinal gradient in this feature, proportional to that in cell length, quite apart from the effects of gibberellic acid. This tendency to constancy of pit-field numbers clearly cannot be wholly true of any one cell at any time, since cell enlargement accompanies cell division, and cells of different lengths, and probably with different histories of cell division, occur adjacent to one another in the same tissue, but within a reasonably uniform tissue it has a general statistical validity. Possibly it is another aspect of the observations of Livingstone (1935) on the characteristic frequencies of plasmadesmata in different types of cell in tobacco. Although as Esau (1953) remarks, the association of plasmadesmata and pit-fields may not be an absolutely constant one, nevertheless some general similarities in the characteristics of their distributions might be expected. In this connexion it has just come to my notice that an increase in the number of plasmadesmata by deposition of cellulose within them was referred to by Priestley (1930), though he did not associate this process with division of the cell. However, it is, as we have seen, not characteristic of the simple enlargement phase, and Priestley was then writing in general of meristem cells.

The maintenance of the number of pit-fields in meristem cell-walls emphasizes their importance as sites of plasmadesmata in the wall; added emphasis of another kind accrues from the recent electron-microscope studies of Whaley *et al.* (1959, 1960) and Porter and Machado (1960), which link plasmadesmata with the endoplasmic reticulum and the nucleus. The complete disappearance of pit-fields in cells of growing onion root-tips, described by Jensen and Ashton (1960), seems to be more apparent than real. Observation with a polarizing microscope shows that they are certainly present in large numbers in the older parts of onion roots, as indeed Scott *et al.* (1956) have described, though their numerical distribution in this tissue has not been studied in detail in relation to growth and cell division.

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Viability of Cereal Seed for Brief and Extended Periods

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With one Figure in the Text

ABSTRACT

Reports of viability periods in cereal seeds of a few minutes or days are discussed in relation to periods of up to 123 years. Attention is drawn to various anomalies which are apparent when relating these extreme periods of viability to previously discussed results in the range of a few weeks to a few years.

INTRODUCTION

IN a previous contribution to this journal (Roberts, 1960) evidence was presented which shows that the life duration of cereal seed, at least over a certain range of conditions, bears a simple relationship to temperature and moisture content and can be related to these conditions, if constant, in the following three ways:

1. The distribution of death points in time is Gaussian (with certain possible exceptions under very moist conditions which were discussed).

2. The half-viability period (i.e. the time taken from harvest for half the seeds to die; this is also the same as the mean viability period of the population) is related to moisture content and temperature as follows,

$$\log p = K_v - C_1 m - C_2 t \qquad (1)$$

where p = half-viability period in weeks, m = percentage moisture content, t = temperature in °C., and K_v, C_1, and C_2 are constants.

3. The spread of the distribution of death points in time is directly proportional to the half-viability period. This can be expressed as

$$\sigma = K_\sigma p \qquad (2)$$

where σ = the standard deviation of death points in time and K_σ is a constant.

These relationships are sufficient to define the pattern of loss of seed viability and to predict theoretically the percentage germination after a given period of time under known constant conditions.

Since it is assumed here, on the basis of previous work, that the curve showing percentage germination plotted against time represents a cumulative normal distribution, mention should be made of the only previous detailed discussion of this curve of falling viability of which the author is aware. Gane (1948), working with Chewing's fescue seed (*Festuca rubra* var. *commutata*) suggested that the curves showing percentage germination plotted against time agreed well with a modified Gompertz equation. Though this

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curve is not symmetrical, by virtue of its sigmoid shape it would in fact provide an approximate fit. But the use of the Gompertz equation in this context is entirely limited to an empirical description of the data, whereas a cumulative normal distribution can be related rationally to factors controlling viability.

The evidence for the relationships outlined in the first paragraph was obtained in the main from results in which the values for the half-viability period ranged from 1 to 150 weeks, although mention was also made of less reliable results obtained over periods of up to 7 years. The data included conditions varying from 11 to more than 25 per cent. moisture content and from 15° C. to 25° C., and a few less-reliable results obtained at temperatures up to 40° C. were also considered. It was pointed out that the applicability of the discovered relationships outside these ranges was not known. The purpose of the present paper is to discuss some evidence which sheds light on the viability relationships in these little-explored regions.

EXPERIMENTAL EVIDENCE

The Distribution of Death Points in Time

The evidence previously given for equation 2 was meagre. Since the validity of this equation needs to be demonstrated for the present discussion, additional evidence is now presented.[1]

Fig. 1 shows some data obtained from 14-day germination tests carried out on Atle wheat stored under various combinations of moisture and temperature in sealed test-tubes. Since harvest and before the experiment started, the seed had been stored in a slightly heated room for between 32 and 42 weeks; as a result, some of the seeds were not viable at the beginning of the experimental period. Because of this, a correction has been applied so as to ignore the proportion of the population which was initially dead. At the beginning of the experiment the percentage germination was about 88; all the experimental values have therefore been multiplied by 100/88 before presentation in Fig. 1. This introduces an error into the interpretation of the results, but, because the preceding loss of viability was small and the rate of loss of viability was also small in relation to the slowest rate of loss in the experimental treatments, the resulting error is also small and does not invalidate the conclusions which have been drawn.

In Fig. 1 the corrected percentage germination has been plotted on a probability scale against time. It will be seen that in all cases the distribution of death points may be considered normal. Two horizontal lines are shown, one at the 50 per cent. level and the other at the 15·9 per cent. level. The distance along the abscissa to the point where a curve showing the percentage germination intersects the 50 per cent. level represents the half-viability period, and it can be shown from the area under a normal curve that the distance from this point to the point where the curve intersects the 15·9 per cent. level is an estimate of the standard deviation (σ). From Fig. 1 it can be demonstrated by the simple geometry of equiangular triangles that the ratio $\sigma : p$ is the same for any of the curves. The value of K_σ calculated from these results is 0·35; but because of limitations described above, this should only be taken as approximate.

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[1] The experimental results which provide this evidence were very kindly made available to me before publication by Miss Mary B. Hyde of the Pest Infestation Laboratory, Slough, England.

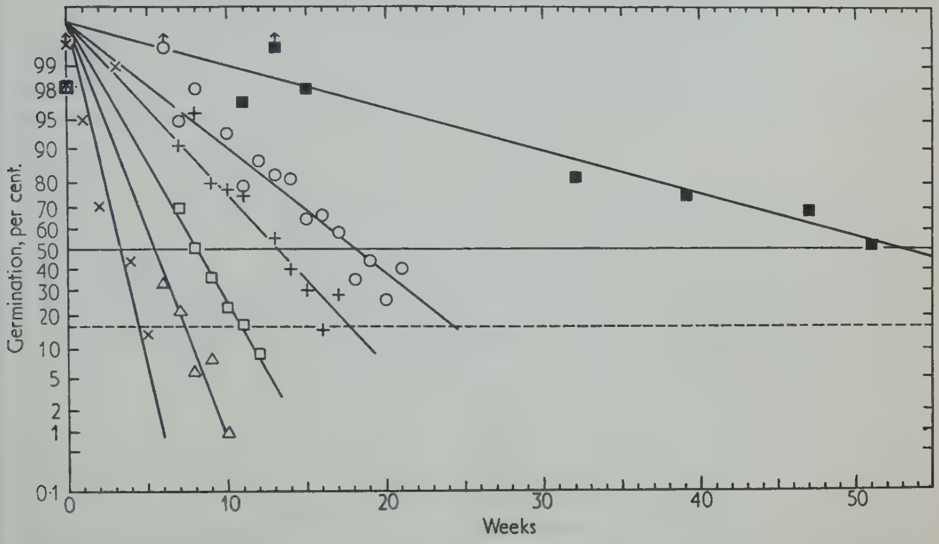

FIG. 1. Corrected values for percentage germination of Atle wheat plotted on a probability scale against time. Since a value of 100 does not appear on a probability scale, where such a value occurs it is represented by a point at 99·5 per cent. and an arrow pointing upwards. Storage conditions were as follows:

	Moisture content, per cent.	Temperature, °C.
×	22·5	25
△	20·5	25
□	18·6	25
+	17·2	25
○	20·6	15
■	17·1	15

Very Short Viability Periods

When the relationship between environmental conditions and viability was discussed previously it is regretted that one notable contribution to this subject was overlooked. This is a paper by Groves (1917) which deals with the effects of relatively high temperatures—i.e. above the normal ambient temperatures—on the viability of wheat seed. To some extent the conclusions drawn were anticipated in a previous note by Crocker and Groves (1915). Groves's paper described experiments in which seeds were treated at constant moisture contents to temperatures ranging from 50° C. to 100° C. for periods ranging from 3 minutes to 13·5 days. From these experiments it was deduced that the relationship between temperature and viability could be described by the following equation which was derived by Lepeschkin (1913) for studying the effect of temperature on the death of plant materials and which was based on the work of Buglia (1909) on the coagulation of proteins:

$$T = a - b \log Z, \tag{3}$$

where T = temperature in °C., Z = the period taken in minutes for 75 per cent. of the seeds to be killed, and a and b are constants. It will be seen that this is the same relationship, but set out in a different form, as that derived (Roberts, 1960) for temperature and half-viability period from data concerned with lower temperatures and which was described in the following way:

$$\log p = k_m - C_2 t, \tag{4}$$

where p = half-viability period, t = temperature, and k_m and C_2 are constants.

Groves did not go on to show the relationship with moisture content, but he must be credited with noting that not only does raising the moisture content at any given temperature decrease the viability period, but at 9 and 12 per cent. moisture content at least the gradient of the curve relating $\log Z$ to temperature was not altered by moisture content: translated into the terms of equation 4, the value C_2 is independent of moisture content. Experiments were also carried out by Groves at 17·5 per cent. moisture content, but the fact that loss of viability occurred in periods as short as 3 minutes at the higher temperatures made such results difficult to interpret since the error due to the time taken for seeds to warm up to the experimental temperature was then large. Nevertheless, the observation, based on the more reliable results, that there is no interaction of moisture content with temperature at high temperatures agrees with the author's conclusions concerning results obtained at lower temperatures over a wider range of moisture contents.

In one respect, Groves's results are difficult to compare with those of other workers. This is because he used a longer germination test than is usual: the actual length of the test is not made clear, but it is evident that it was of at least 20 days' duration. Most other workers employ a shorter period, and this has important consequences. In populations of most cereal seeds which show a high percentage viability, when appropriate conditions are given, germination is normally completed within 3 or 4 days; but in populations which show a low percentage viability, most of the seeds which germinate do so at a very much slower rate and some may take 2 or 3 weeks. This is a common experience of seed testers and is also evident in the few detailed results which Groves published. A further complication is raised by the tendency for seeds in the germination test which have not germinated after about 10 days to be destroyed by micro-organisms (Müller, 1913) unless the test is carried out under relatively sterile conditions. (Groves got over this difficulty by sterilizing the seed in silver nitrate and using sterile Petri-dishes.) Therefore, although either a short or a long test will give the same result when the percentage viability is high, the longer test gives the more accurate result when the percentage viability is low provided it is carried out under sterile conditions. But it does not necessarily give the most useful result since, from the practical point of view, one is normally more interested in ability to germinate under more natural conditions. By the time germination has dropped to 50 per cent., a distinct difference in percentage germination could be expected depending on the duration of the germination test, and by the time 75 per cent. are dead—the end-point used by Groves—a somewhat greater difference could be expected. An analysis of the detailed example of germination results published by Groves suggests, for instance, that his germination test would give an increase of about 23 per cent. in half-viability period when compared with the result of a 7-day test.

But in spite of this difference, in terms of equation 4 the value of the constant C_2 should not be affected by the length of the germination test, though the value of k_m would be altered. A measure of the rate of loss of viability is given by the reciprocal of the time taken for percentage germination to drop to a given end-point. The temperature coefficient (Q_{10}) can then be calculated by comparing rates at different temperatures. For the purpose of calculating the Q_{10} it follows from equation 2 that it is immaterial which end-point is chosen provided it can be accurately determined: the half-viability period which the author has used or the time taken for germination to drop to 25 per cent. as used by Groves should give the same Q_{10}. Since it is the constant C_2 which controls the gradient of the curve relating the log end-point to temperature and the value of this is not affected by the length of the germination test, the Q_{10} as calculated by Groves should be legitimately comparable with that reported by Roberts (1960).

Groves in fact reported various values for the Q_{10} because he determined the coefficient from individual pairs of results rather than from his calculated regression lines; but when allowance is made for the sampling error involved in his method it becomes obvious that all his results (except the doubtful ones at the high moisture content) agree well with a Q_{10} of just under 10. This value is much higher than was reported (Roberts, 1960) for results with lower temperatures (3·3) and suggests that the value C_2 as calculated at ordinary climatic temperatures is not applicable at much higher temperatures. It is clear that this aspect needs further investigation.

Very Long Viability Periods

Data concerning the viability of seeds after very long periods where the conditions of storage can be defined are rare; but a particularly interesting case has recently been reported by Aufhammer and Simon (1957). In 1955 some samples of various species of seeds were found sealed in glass tubes within the foundation stone of the Nuremberg City Theatre. A document in the Nuremberg City Archives states that the seeds had been placed there in 1832. When the seeds were removed after 123 years of storage, moisture contents were determined and germination tests (of 5 days' duration) were carried out. The results for the cereals are shown in Table 1.

The temperature conditions of storage are not known but can be inferred to some extent from meteorological data. No figures for soil temperature are available for Nuremberg itself but Dr. U. Simon has kindly supplied figures for the mean monthly soil temperatures at a depth of 50 cm. for Buchenbühl which is a suburb of Nuremberg. These are shown in Table 2.

TABLE 1

Moisture Content and Germination of Cereal Seeds found in the Foundation Stone of the Nuremberg City Theatre (from Aufhammer and Simon, 1957)

	Moisture content of seeds, per cent.	No. of seeds tested	No. of seeds germinated	Percentage germination
Wheat	8·28	20	0	0·0
Oats	8·04	32	7	21·9
Barley	7·30	25	3	12·0

TABLE 2

Mean Monthly Soil Temperature (°C.) at a Depth of 50 cm. at Buchenbühl

Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean
−1·3	0·0	3·4	7·6	12·8	16·1	17·6	16·7	13·3	8·2	3·5	0·1	8·2

Equation 1 was developed from data concerned with seed stored under constant environmental conditions, but there would appear to be no objection to using it for varying temperatures if one assumes that temperature fluctuation has no special effect. Providing the temperature changes occur in cycles which are small in relation to the half-viability period, it should be possible to calculate a figure for the 'effective temperature'—i.e. a value for t which can be substituted in equation 1. Since the half-viability period decreases exponentially with increase in temperature, the arithmetic mean would be unsuitable as the effect of the higher temperatures would be underestimated. The following expression would be appropriate for calculating the effective temperature:

$$T_E = \frac{\log\left\{\frac{\sum[\text{antilog}(tC_2)\times w]}{100}\right\}}{C_2}, \tag{5}$$

where T_E = effective temperature, t = temperature in °C., w = percentage of time each temperature (t) was given, and C_2 is the constant (0·05) from equation 1.

If each temperature (t) is given for the same length of time, equation 5 may be simplified to the following:

$$T_E = \frac{\log\left\{\frac{\sum[\text{antilog}(tC_2)]}{n}\right\}}{C_2}, \tag{6}$$

where n = the number of different temperatures (t) given.

Using equation 6, the effective soil temperature for Buchenbühl is found to be 10·6° C. This temperature and the moisture contents given in Table 1 were substituted in equation 1 using values for the constants previously estimated for wheat ($K_v = 4{\cdot}222$, $C_1 = 0{\cdot}108$, $C_2 = 0{\cdot}050$) in order to determine the value of p. The theoretical periods for germination to drop to the values shown in Table 1 were then calculated by using this value of p in conjunction with equation 2 ($K_\sigma = 0{\cdot}35$ from the data in Fig. 1) and tables showing the area under a normal curve. The theoretical periods were found to be 16·2 years for oats and 21·7 years for barley. The question of the theoretical period for germination to drop to 0 per cent.—the observed germination in wheat—is not straightforward, but the time taken for germination to drop to 5 per cent. would be 19·0 years. Thus using a sample of 20 seeds one could expect to obtain a value of 0 per cent. frequently after this period.

It will be seen that, with the possible exception of wheat, the cereal seeds in the foundation stone maintained their viability for much longer than would have been predicted from results obtained under conditions where the half-viability period is of the order of a few weeks or years. The foundation stone was on the north side of the building and 1·5 metres below the ground (Dr. U. Simon, private communication). These conditions would have certainly made the actual temperatures lower and the fluctuations less than shown in Table 2; thus the effective temperature was probably less than has been calculated here. But even when allowance has been made for this, for the high sampling errors involved in using small numbers of seeds in the germination tests, and for the fact that by the nature of equation 1 slight errors in any of the other values would introduce large errors into the value of p when dealing with long periods, it still seems safe to assume that the equation and the constants given underestimate the percentage viability over very long periods. From the practical point of view, at least, this is reassuring as it means that data which are obtained from periods of the order of weeks can be safely used for designating suitable storage conditions for very long periods. From a theoretical point of view, however, the anomaly is somewhat disconcerting; but perhaps it would be advisable to postpone further discussion until a greater number of results carried out under controlled conditions are available.

It is interesting to note that from his own results with extremely short viability periods Groves tentatively predicted the retention of viability over very long periods. These are two of his examples: at 9 per cent. moisture content and 20° C. germination would drop to 25 per cent. after 111·2 years, and at the same moisture content at 0° C. after 938·5 years. It will be seen that judged by the Nuremberg data, his extrapolations overestimate the ability of seed to retain viability over very long periods.

SUMMARY

1. Available results suggest that the Q_{10} for the rate of loss of viability in wheat seed stored at temperatures above 50° C. is higher than for seed stored

at temperatures in the region 15° C.–30° C. The evidence also suggests that the Q_{10} over the range 50° C.–100° C. is approximately constant.

2. Data for 123 years' storage show that retention of viability in temperate cereal seeds is greater than would be expected from the results obtained over a few weeks or years of storage. Although further results would be necessary to confirm this conclusion, these data indicate that the equations previously suggested for describing the relationship between temperature, moisture content, and viability can be used with confidence in designing conditions for long periods of storage as they tend to underestimate the retention of viability over very long periods.

ACKNOWLEDGEMENTS

I am deeply grateful to Miss Mary B. Hyde for allowing me to use some of her experimental results on storage of wheat seed and to Dr. U. Simon for providing me with data for estimating the temperature of the foundation stone of the Nuremberg City Theatre. This paper is published with the permission of the Director of Agriculture, Sierra Leone.

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The Viability of Rice Seed in relation to Temperature, Moisture Content, and Gaseous Environment

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With three Figures in the Text

ABSTRACT

The seed viability of a tropical variety of rice has been investigated in hermetic storage under a range of conditions of temperature and moisture content. The results confirm a previous suggestion, which was made on the basis of data from temperate cereals, that the relationship between these factors and viability can be described in simple mathematical terms. In addition, a comparison has been made of the effects on viability of hermetic storage in air, oxygen, nitrogen, and carbon dioxide.

INTRODUCTION

IT has previously been shown (Roberts, 1960) that a simple mathematical relationship can be established between temperature, moisture content, and the viability of cereal seeds. The evidence for this relationship was obtained in the main from the common temperate cereals. The salient literature on rice was reviewed and although it was found that there was sufficient information to show that the same type of relationship probably held for this cereal, it was pointed out that the available data were insufficient to calculate the constants applicable to the equations describing this relationship.

The main purpose of the investigation described here was to confirm whether in fact the indicated equations do apply to rice seed and if so, to determine the relevant constants. At the same time an attempt has been made by appropriate design of experiment to place the general concept of viability in cereal seeds on a sounder basis. In particular, special attention has been paid to the effect of temperature, since the evidence in temperate cereals is not especially strong in this respect; on the other hand, it has not been considered necessary to investigate the effects of moisture content in such great detail since the evidence concerning this aspect in other cereals was shown to be sufficiently convincing. However, in rice, results (Kondo and Okamura, 1930*a*) which were previously discussed showed anomalous behaviour between 14 and 16 per cent. moisture content. The moisture contents used in the present experiments were therefore chosen to include this range. In choosing a range of temperatures for investigation particular attention has been paid to the need for information where least knowledge exists and previous discussions (Roberts, 1960, 1961) would lead one to suspect that complications might be found, i.e. between 30° C. and 50° C.

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In addition, an investigation into the effects of the common gases present in air has been included. This is intended mainly as a preliminary study into the effects of hermetic as compared with open storage.

MATERIAL AND METHODS

The term seed is used here in the agricultural sense and refers to the caryopsis surrounded by the attached lemma and palea. The seed used for the present investigation was of the variety Toma 112 (*Oryza sativa* L., subspecies *indica*) which is a pure-line selection derived from indigenous material in Sierra Leone. A sample was harvested and sun-dried for a period of 2 days. The seed was frequently mixed and turned during the drying process and three sub-samples were taken at different times. The samples were stored in air-tight jars until the 3rd day after harvest when the moisture contents were determined by drying the seed, after grinding, in a ventilated oven at 105°C.±1° C. for 24 hours; their moisture contents, expressed as a percentage of the weight before drying, were 14·5, 13·5, and 12·0 per cent.

On the 3rd day after harvest the sub-samples were distributed among small hermetically sealed containers at the rate of approximately 130 seeds per container. In some treatments the seeds were stored in air; the containers used in these cases were miniature screw-capped bottles with waxed-rubber seals having an internal volume of approximately 7·5 ml. In other treatments where the seeds were stored in single gases—oxygen, nitrogen, and carbon dioxide—glass ampoules were used with an internal volume of approximately 8 ml. The ampoules were constructed from 8-mm. bore soda-glass tubing and after filling with seed they were drawn out to a narrow bore at both ends. The appropriate gas was then passed through the ampoule for about 20 seconds and the ends of the ampoule sealed in a flame before being removed from the gas-supply line. Oxygen and nitrogen were supplied from cylinders under pressure. Carbon dioxide was prepared in a Kipp's apparatus and was dried by passing through calcium chloride.

After filling, the ampoules or bottles were placed in one of five incubators, ranging from 27° C. to 47° C. in 5° C. intervals, according to the temperature of the treatment. None of the incubators varied by more than 1° C. from their nominal temperature during the course of the experiment. Temperature gradients of as much as 2° C. existed in some incubators, but an effort was made to minimize the errors due to these gradients by careful packing within the incubators.

Germination tests were carried out on all treatments at regular intervals; the interval varied between treatments and depended on the rate of loss of viability. The contents of one bottle or ampoule were used for each germination test which was carried out as follows. One hundred seeds were distributed equally between four 9-cm. Petri-dishes. The bottom of each dish was lined with one 9-cm. circle of Whatman No. 1 filter-paper and irrigated with 10 ml. distilled water. The seeds were incubated for 7 days at 32° C.±2° C. During incubation 50 of the seeds were kept in darkness by enclosing two of the Petri-dishes in a cardboard box; the remaining 50 seeds in the other two Petri-dishes were subjected to diffuse daylight of low intensity (of not more than about 300 lux) which was allowed to enter the incubator through the inner glass door as the outer door was removed. The date of each germination test was recorded as the date on which the test started. A seed was considered to have germinated if by the end of the test the radicle and coleoptile protruded from the seed by more than 2 mm.

In presenting the results, the data from the light and the dark Petri-dishes have been summed, since no consistent effect of light could be shown. This observation is different from that of Sircar and Biswas (1960) who in discussing viability reported increased germination in white light. In this laboratory a distinct effect of light on percentage germination has only been found when the population of seeds is partially dormant; white light then causes some of the seeds to loose their dormancy.

RESULTS

Seed sealed in Air

The results of the treatments where the seeds were sealed in air are shown in Fig. 1. Theoretical curves showing the falling viability plotted against time have been constructed on the assumptions discussed previously (Roberts, 1960, 1961) that the distribution of death points is Gaussian and that the following equations apply:

$$\log p = K_v - C_1 m - C_2 t \quad (1),$$

where p = half-viability period (i.e. the mean period of viability of the population) in weeks, m = percentage moisture content, t = temperature in ° C., and K_v, C_1, and C_2 are constants;

$$\sigma = K_\sigma p, \tag{2}$$

where σ = the standard deviation of death points in time and K_σ is a constant.

The values assigned to the constants in constructing the curves shown in Fig. 1 were $K_v = 5{\cdot}686$, $C_1 = 0{\cdot}159$, $C_2 = 0{\cdot}069$, and $K_\sigma = 0{\cdot}21$. It will be seen that within the limits of experimental error the theoretical curves provide a good fit to the experimental data.

In order to provide what may be a clearer general picture, the theoretical relationship has also been presented in another form in Fig. 2.

Seed sealed in Single Gases

One of the advantages in the experimental technique used in this study is that hermetic sealing provides a simple and reliable method of controlling the moisture content of the seed. However, this advantage is obtained at the expense of causing alterations in the gaseous composition of the atmosphere surrounding the seeds; this is due to the fact that at the moisture contents used, respiration of the seed and associated micro-organisms takes place (Milner and Geddes, 1954) and thus the oxygen concentration will decrease while that of carbon dioxide will increase. But if the seed is sealed in nitrogen or carbon dioxide instead of air, then changes in the gaseous environment during the course of the experiment are likely to be less. On the other hand,

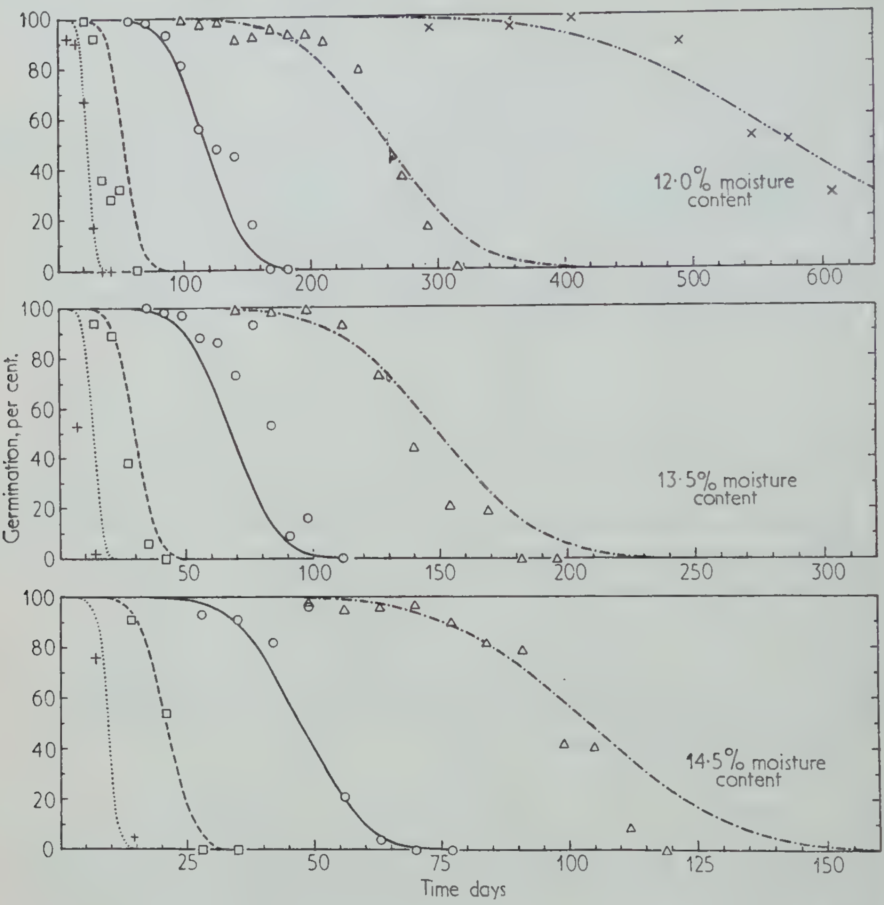

FIG. 1. Loss of viability in seed of the rice variety Toma 112 hermetically sealed in air at different temperatures and moisture contents. Note that the time scale is different for each moisture content: reading from top to bottom, each scale is a 2 × magnification of the previous one. Storage temperatures were as follows: 27° C. (×), 32° C. (△), 37° C. (○), 42° C. (□), 47° C. (+). The curves are theoretical; the method of calculation is described in the text.

the use of oxygen would result in greater changes than the use of air. Although none of these treatments would be equivalent to using open storage where the gaseous environment would be relatively constant, it was thought that their use would shed some light on the possible differences between hermetic and open storage, particularly with regard to possible deleterious effects of either oxygen or carbon dioxide. Furthermore, from the practical point of view, these treatments should provide data as to whether any of the gases investigated could be usefully employed in extending the viability period of seeds.

The results of the single-gas treatments are presented in Fig. 3. The corresponding results obtained in air are also included for comparison. It will be seen that under the conditions of the experiment, the differences between the gas treatments, although distinct, are not very large. Detailed interpretation of the results is difficult since the effect of a given gas on viability is not always the same at all temperatures and moisture contents. Nevertheless, it may be concluded that when other conditions are favourable for maintaining viability, storage in nitrogen increases longevity. The same effect was not shown for carbon dioxide which was found to be either worse or no better than air.

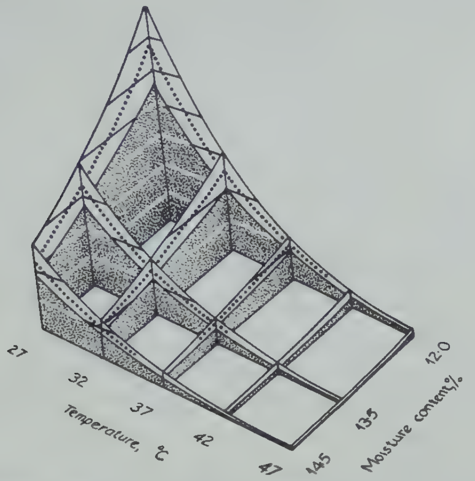

FIG. 2. Perspective drawing of a three-dimensional graph showing the theoretical relationship between temperature, moisture content, and viability in the rice variety Toma 112 over the range of conditions investigated. The vertical scale represents time: the horizontal lines indicate 100-day intervals. The dotted line represents the half-viability period (for clarity, this has been omitted at the higher temperatures). The unshaded areas represent this value ± the standard deviation of death points in time; thus it follows from the area under a normal curve that the line at the top of the shaded areas represents the time taken for germination to drop to 84·1 per cent., and the line at the top of the unshaded portion represents the time taken for germination to drop to 15·9 per cent.; 68·2 per cent. of the seed may be expected to die within the unshaded region.

Without further confirmation, it would be unprofitable to discuss the possibilities of interactions which the results indicate.

DISCUSSION

The applicability of the present results to long-term storage. In the present experiments, viability was investigated in seeds stored for periods up to little more than $1\frac{1}{2}$ years. It is interesting to note, however, that using the constants determined from this investigation, good agreement is shown with a result for rice storage for a period of 9 years (the longest period of which the author is aware when rice seed has been stored under controlled conditions).

Teresaka (1941) noted that a sample of rice stored at 10° C. and 13·8 per cent. moisture content was 90 per cent. viable after 9 years. From the present experiments, 90 per cent. germination would be predicted to occur under these conditions after 8·89 years. Although this remarkable agreement suggests that the relationships described here still hold for conditions well outside the range of those investigated, not too much reliance can be placed on this comparison since, as will be discussed later, there is evidence that there are varietal differences in ability to retain viability.

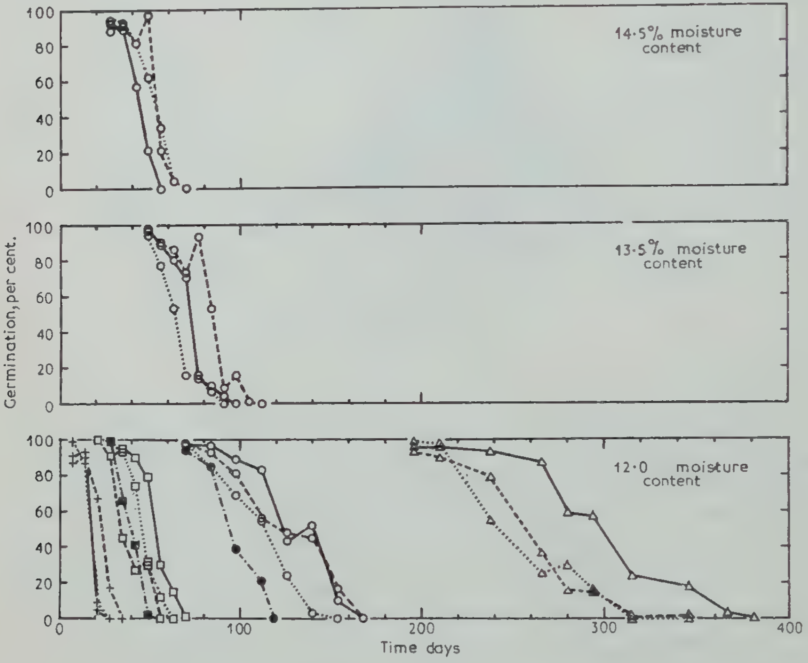

FIG. 3. Loss of viability of seed of Toma 112 hermetically sealed in various gases: nitrogen (————), air (– – – –), oxygen (······), carbon dioxide (■ ● –·–·–·). Storage temperatures: 32° C. (△), 37° C. (○), 42° C. (□), 47° C. (+).

Temperature. The results described here are considered to support the proposition that the viability of cereal seeds bears a simple and definable relationship to temperature and moisture content. But the results have also served to emphasize several points which require further investigation. Perhaps the most important of these concerns the temperature coefficient. In the region of 15° C. to 20° C. it was shown (Roberts, 1960) that the Q_{10} for loss of viability in wheat is about 3·3. Groves (1917) found that a much higher Q_{10} was applicable to wheat in the range 50° C. to 100° C. and examination of his evidence (Roberts, 1961) indicates that a constant Q_{10} of about 10 is shown over this range. From these results it would follow that a change in Q_{10} must occur between these two ranges of temperature. The choice of the temperature range used in the present investigation was partly governed by this suggestion; but the results, although showing a Q_{10} between the values just discussed, i.e. 4·9, do not provide evidence which suggests a change in the value over the range 27° C. to 47° C. To clear up these anomolies, it would be necessary to investigate within one experiment a very much wider range of temperatures than has so far been attempted.

Gaseous environment. Suggestions have often been made (e.g. Curtis and Clark, 1950; Crocker and Barton, 1953; Milner and Geddes, 1954) that anaerobic conditions lead to rapid loss of seed viability. On the other hand it has also been suggested that anaerobic conditions should reduce loss of viability (Teunisson, 1954) and beneficial effects on longevity have been claimed for anaerobic conditions—e.g. in rice (Sahadevan, 1953), and in onion seed stored in carbon dioxide (Harrison and McLeash, 1954). Toole *et al.* (1948) found no deleterious effects of storing a wide variety of seeds in small glass vials when compared with open storage. But in many cases, such as those mentioned above, it is difficult to draw definite conclusions concerning the effect of anaerobic conditions since other factors which affect viability, such as moisture content, have not been kept the same in the aerobic and anaerobic treatments. An examination of the short review on gas storage by Owen (1956) confirms our lack of critical data on this subject. Because of the difficulties of interpretation, no mention will be made here of much of the work on storage of seed under sealed and unsealed conditions; but it will be useful to consider some work where the storage environment has been closely controlled.

In some experiments on Chewing's fescue seed (*Festuca rubra* var. *commutata*), Gane (1948) concluded that in storage for periods of as long as 4 years there was little to choose between storage in air or nitrogen under conditions where temperature and moisture were closely controlled. In these experiments the seeds stored in nitrogen were contained in sealed tubes; those in air were kept in desiccators above solutions of sulphuric acid, but it is not clear whether desiccators were hermetically sealed or not, though it seems probable that they were. In some recent work on wheat, Glass *et al.* (1959) stored seed under conditions where not only were temperature and moisture maintained constant but also the gaseous composition of the atmosphere. In one trial at 30° C. no difference was shown between the effects of air and nitrogen in the range 13 to 18 per cent. moisture content, but in a second trial nitrogen was shown to be slightly more effective for retaining viability. In a trial at 20° C. storage in nitrogen was shown to cause a distinctly greater retention of viability, particularly at a higher moisture content. Peterson *et al.* (1956), also using wheat seed, showed that in short trials at 30° C. and 18 per cent. moisture content in controlled oxygen-nitrogen mixtures, decreasing the oxygen content led to an increased germination percentage when tested after 16 days' storage. In mixtures containing 21 per cent. oxygen and variable amounts of nitrogen, increasing the carbon dioxide content also led to an increase in percentage viability. Kondo and Okamura (1930*b*) compared hermetic storage of rice at similar moisture contents (between 11 and 12 per cent.) in air and in carbon dioxide at ambient temperature. Over a period of 4 years they showed that viability did not drop below 93 per cent. in any treatment. Their results do not allow definite conclusions to be drawn concerning the relative merit of carbon dioxide as a medium for storage, but they do emphasize the fact that it cannot be markedly deleterious.

When considering the results reported in this paper in relation to the other work which has been discussed, it is evident that some of the data are conflicting; this suggests the existence of problems which have not yet been fully exposed. Nevertheless there is general agreement on the main points. Anaerobic conditions do not appear to be deleterious to the viability of cereal seeds. Under most conditions, storage in nitrogen results in a slightly longer period of viability than storage in air under similar conditions of temperature and moisture. The fact that increases in oxygen concentration tend to decrease the viability period also suggests that aerobic conditions are not specially beneficial to maintenance of viability; though it is also possible that the type of respiration is unimportant and that oxygen has some other effect: for instance, high oxygen tensions have been shown (Kronstad *et al.*, 1959) to have a mutagenic effect on barley seeds. The effect of carbon dioxide in high concentrations is not entirely clear: in some cases it appears to have been better for maintaining viability than nitrogen and in other cases worse; but in any case it is certainly not markedly deleterious to viability.

Whether storage in nitrogen—or other inert gas—as a practical measure for seed storage would be worth the special methods which would be necessary seems doubtful. In our present state of knowledge it is suggested that it would generally be more profitable to concentrate on lowering the moisture content and temperature of the seed.

Genotype. Finally, there remains the question of the similarities and differences in seed viability between different genotypes. It was pointed out (Roberts, 1960) that the available evidence, although not substantial, suggests that different varieties of wheat behave similarly. Furthermore—and here the evidence was more convincing—the available data also indicate that the viability of oats and barley can be described in terms of the same constants as wheat. The results described here show that in at least one variety of rice the viability constants are different from these other cereals and the half-viability period is greater under any given set of conditions. In much of the published work on rice viability the experiments have been carried out under conditions which have not been closely controlled; but there are many suggestions at least that varietal differences exist in the ability to retain viability (e.g. Jones, 1926; Sahadevan, 1953; Mudalier and Sundararaj, 1954; Dore, 1955; Ghose *et al.*, 1956; Ananthakrishna Rao, 1959). Preliminary observations in this laboratory have indicated that there may be differences particularly between the *indica* and *japonica* sub-species. Thus it would be inadvisable at present to use the results described here for predicting the viability of all rice varieties, though they can probably be used as a useful guide for storing *indica* varieties.

SUMMARY

1. The retention of viability of one variety of the *indica* sub-species of *Oryza sativa* L. has been investigated in hermetic storage. It is shown that under a wide range of conditions the percentage germination can be related to temperature and moisture content by simple equations. The constants in the equations applicable to rice are not the same as have been calculated for wheat: they are such that rice seed may be expected to show a greater period of viability under any given set of conditions.

2. A comparison of the effects on viability has been made between hermetically sealing the seeds in air, oxygen, nitrogen, and carbon dioxide under a range of conditions of temperature and moisture content. The results are complex and it is evident that the influence on viability of one gas as compared with another is not necessarily the same under all régimes of temperature and moisture. When the period of viability is of the order of a year, there is evidence that storage in nitrogen may lead to an increased period of viability when compared with storage in air; this result is in general agreement with other work which has been carried out on the storage of wheat seed. It is doubtful, however, whether the increase due to nitrogen is sufficiently large to warrant its use as a practical measure for storage of cereal seed.

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Inequality in the Development of the Axillary Members in *Glechoma hederacea* L.

BY

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With seven Figures in the Text

ABSTRACT

The vegetative plant of *Glechoma hederacea* has leaves and axillary buds arranged in opposite and decussate pairs. The two individuals of any pair of axillary members (i.e. buds or runners) are often unequal in size although the leaves subtending the particular pair of axillary members seldom show any obvious inequality. A quantitative investigation of this inequality of the axillary members was made on clonal material. The lengths of the pairs of axillaries at 191 nodes on 25 horizontally growing runners (stems) were recorded. The degree of inequality of one member of each pair of axillaries on a number of these runners was calculated as its percentage difference from the mean length of the particular pair of axillary members. The positions of the axillaries on the runners were relatable to the four orthostichies present on the axillary buds from which the runners developed. At the even nodes (i.e. the second, fourth, &c. formed nodes) there was always a considerable degree of inequality between the members of any particular pair of axillaries. The longer members invariably occurred on the orthostichy of the runner which in the undeveloped axillary bud lay adjacent to its subtending leaf. At the odd nodes the inequality between the members of any pair of axillaries was usually not so great and occasionally the members were equal. Although the longer axillaries were not confined to any particular orthostichy there was often a tendency for more to occur on one of the two possible orthostichies on any given runner.

Fifteen terminal runner buds were grown vertically in narrow-bore glass tubes for a varying number of plastochrons. At the 67 nodes at which it was certain that the pairs of axillary members were initiated after the beginning of the experiment a similar pattern of inequality was found to that occurring in the horizontal runners. It is thus concluded that gravity plays no part in determining such inequality.

A comparison was made between the degree of inequality of one of the individuals of each axillary pair at a total of 18 nodes on four horizontal runners and the inequality (calculated from laminal areas) of the leaves subtending these axillaries. The degree of inequality of the subtending leaves was usually much less than that of their respective axillaries and bore no constant relationship to the latter.

INTRODUCTION

RECENTLY White (1955 and 1957) discussed the phenomenon of anisophylly, or inequality in size between the two members of a pair of leaves, in plants with a decussate phyllotaxy and with special reference to young plants of *Acer pseudoplatanus* L. The similar occurrence of inequality between

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the two members of a pair of axillary buds in plants with a decussate leaf arrangement was recorded by Goebel (1900, p. 111) and more recently discussed by Champagnat (1948) and Shushan and Johnson (1955).

In *Glechoma hederacea* the leaves and axillary members (i.e. buds or shoots) are arranged in opposite and decussate pairs, and although some anisophylly occurs in the vegetative plant it is not usually very evident. However, inequality in length between the two axillary members at a given node is frequently very marked even at early stages in their development (Fig. 1). This inequality was found both in the clonal material on which the results of this investigation are based and also in other non-clonal plants grown under similar conditions. An attempt has been made to investigate the inequality of the axillary members in *Glechoma* on a quantitative basis and to discuss some of the possible factors influencing this phenomenon.

MATERIAL

The material used in this study was clonal, being derived from a single plant collected in the field during October 1957. When collected the material showed little active growth but on examination many of the shoot apices were found to be reproductive and forming lateral flower primordia. However, in a warm green-house, these apices quickly reverted to the vegetative condition. The clonal material derived from this plant has remained vegetative for the last three years under these conditions. Since the vegetative plant has a prostrate habit it was grown on large horizontal benches covered with several inches of soil and the runners which root at the nodes could thus develop freely.

During autumn and winter the green-house was heated and the lighting supplemented for part of the time by the use of mercury vapour lamps. Nevertheless, during these seasons there was little active growth of the axillary buds, although the growth of the runners continued vigorously. In spring and summer this apical dominance was not nearly so obvious on the runners and the axillary buds developed freely, but there was still a tendency for these axillaries to be less vigorous than the parent runners. If at any time the terminal bud was removed then the pair of buds at the youngest node left on the runner quickly and vigorously developed. The pair of runners which thus formed showed the same pattern of apical dominance as their parent runner. Nearly all of the axillary runners used in this investigation were stimulated to grow by decapitation of the parent runners.

PRELIMINARY OBSERVATIONS

Fig. 2A is a plan showing the topography of an axillary bud, and its relationship to the parent axis and subtending leaf on a horizontal runner, when viewed from behind. There are four orthostichies present in such a bud and the first formed pair of leaves are termed upper and lower relative to the bud axis and lie at right angles to the plane of the parent leaf axil. The second pair of leaves lie on the left and right respectively of the bud axis and in the plane of the parent leaf axil. Second order bud primordia are present in the axils of the leaves on the axillary bud very soon after the development of these leaves.

As the axillary bud develops the young nodes (and their associated leaves and axillary buds) become successively separated from the terminal runner bud (Fig. 2B). A mature node is defined as any node situated on a runner behind the terminal bud of that runner. The internodes of such a runner tend to be considerably twisted, but since the stem is four-sided it is possible to untwist it and to relate the positions of the leaves or axillary members at any particular node to one of the four orthostichies of the axillary bud from which the runner developed.

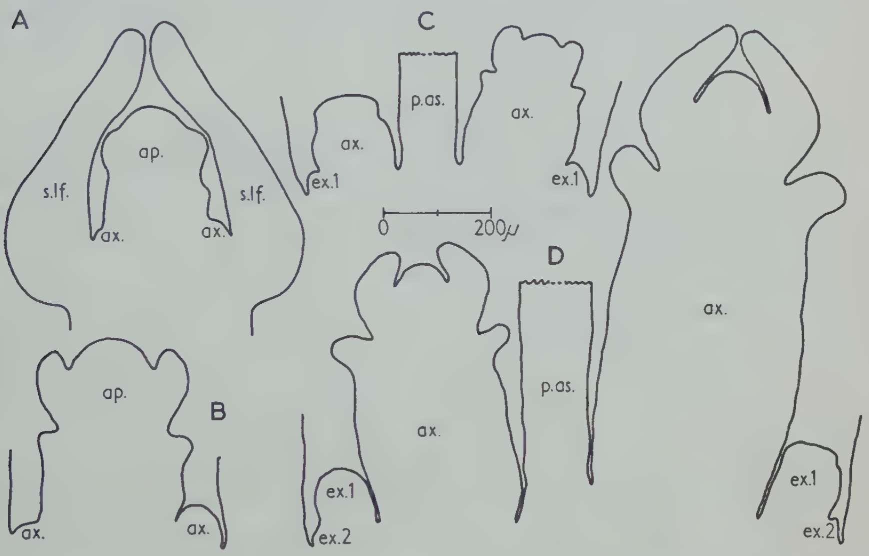

FIG. 1. Pairs of axillary buds at various stages of development present at young nodes of cleared terminal buds. The larger axillary of a pair has always been drawn on the right of the parent axis. A, the buds still developing from the vegetative apex of the terminal bud. B, a slightly later stage, only the lower limits of the adaxial faces of the subtending leaves are shown. In both C and D the width of the parent axis between the axillary buds has been greatly reduced. In C the first-order extra-axillaries are developing and in D the second-order extra-axillaries are just visible. *ap.*, apex of terminal bud; *ax.*, axillary bud; *ex. 1*, first-order extra-axillary bud; *ex. 2*, second-order extra-axillary bud; *p.as.*, parent axis; *s.lf.*, subtending leaf.

During the early development of an axillary bud an additional, or extra-axillary bud, develops from the base of the axillary bud on the side adjacent to the subtending leaf (Fig. 1C, ex. 1). Additional extra-axillary primordia may form in relation to the same axillary bud: each successive one developing from the base of the last formed extra-axillary primordium on the side adjacent to the subtending leaf of the axillary bud (Fig. 1D, ex. 2). Especially in summer the first formed order of extra-axillary buds may develop into normal vegetative runners but under normal conditions this rarely happens to the next order extra-axillaries.

A preliminary study of the inequality of the axillary members was carried out on two pairs of runners. At each of the nodes a visual estimate was made as to the lengths of the axillaries and the orthostichies of the longer members were recorded. There were 28 mature even nodes, i.e. the second, fourth, &c. formed nodes of these runners (Fig. 2B, nodes 2, 4, &c.) and 28 odd nodes, i.e. the first, third, &c. formed nodes of the runners, at which both axillary members were intact. At the even nodes of the two left runners the longer axillaries were always on the left orthostichies and on the two right runners the longer axillaries were always on the right orthostichies. Thus on all four

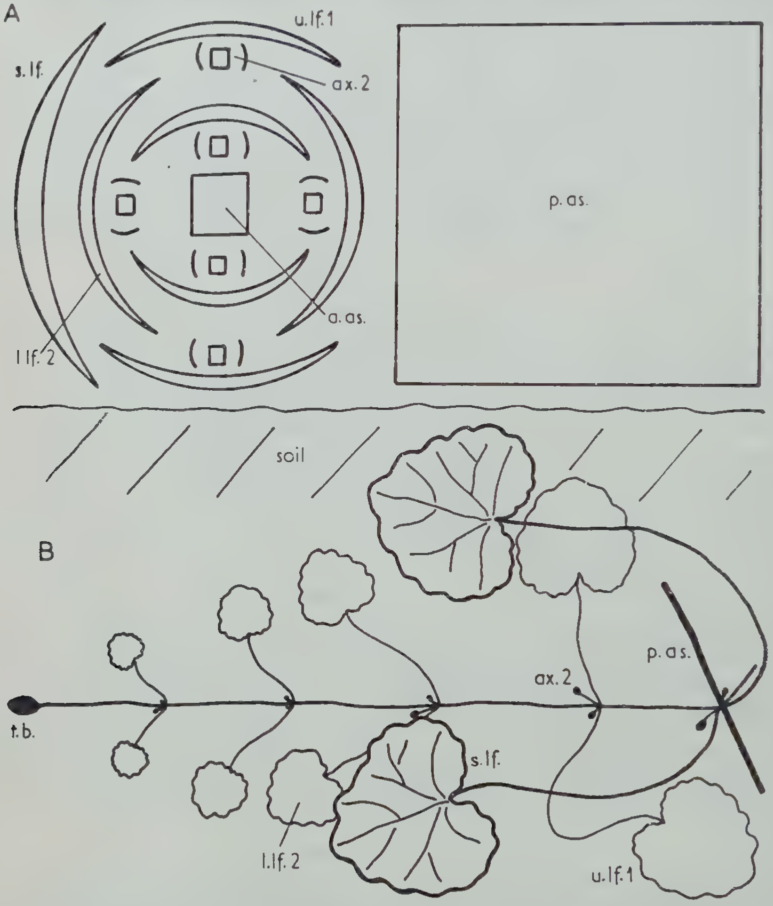

FIG. 2. A, plan of an undeveloped axillary bud viewed from behind showing its relationship to the parent axis and subtending leaf. B, diagram of the axillary runner formed when the bud shown in A develops. *a.as.*, axis of axillary bud; *ax. 2*, second-order axillary bud., *l.lf.2*, left leaf at node 2; *p.as.* parent axis; *s.lf.*, leaf subtending first-order axillary member; *t.b.*, terminal bud of runner; *u.lf.1*, upper leaf at node 1.

runners the longer axillaries at the even nodes always occur on the orthostichies which were adjacent to the subtending leaves of the axillary buds from which the runners developed (Fig. 2A). At the odd nodes of the two left runners eight longer members were found on the upper orthostichy and six on the lower. On the two right runners one longer axillary was found on the upper orthostichy, twelve were on the lower, and at one node the upper and lower members were more or less equal in length. There is thus no such constancy in the arrangement of the axillaries at the odd nodes of the runners as there is at the even nodes.

FURTHER OBSERVATIONS

Ten pairs of runners and three single runners of varying length, with a minimum of four and a maximum of twelve mature nodes present, were examined together with the terminal portions of two other well developed runners. The lengths and orthostichies of the axillary members at each mature node were recorded. In addition, measurements of the lengths of the axillary buds within some of the terminal buds of these runners were made, employing a clearing technique in 5 per cent. sodium hydroxide (Bowes, 1960). In view of the great variability in length of the axillary members (ranging from just over 10μ to nearly 30 cm.) a variety of scales was employed. All measurements over 1 cm. were made with a millimetre rule. Those beneath this range were taken by using different magnifications of a binocular or monocular microscope and projecting the image of the bud by camera lucida on to the relevant scale and measuring the length direct in microns. Where measurements were made of axillary buds within the cleared terminal buds (Fig. 1 shows various stages of such axillary buds present within the terminal bud) it was essential for the correct orthostichies of these axillary buds to be known. Such information was easily available if, before removal from the runner, the orthostichies of the oldest pair of leaves of the terminal bud were noted. The laminas of this oldest pair of leaves were removed and one petiole cut shorter than the other and it was thus possible to distinguish between the two orthostichies in the stained mount of the terminal bud.

Table 1 records the lengths in millimetres of the axillary members on the four orthostichies of each member of a pair of axillary runners. The axillary buds at nodes 11–13 and 10–12 of the left and right runners respectively were present in the cleared terminal buds of these runners. The degree of inequality of each of the left axillary members at the even nodes, and the upper axillaries at the odd nodes, has been calculated as the respective percentage differences of each of these members from the mean length of the particular pair of axillary members concerned. By this method of calculation the sum of the inequalities of the two members of any pair of axillaries is always 200 per cent. It follows therefore that the percentage inequality of the shorter member is always equal to the difference between the percentage inequality of the longer member and 200 per cent. The values for the degree of inequality of the left or upper axillary members have also been expressed as the individual

TABLE 1

Lengths of Axillary Members on the Four Orthostichies of a Pair of Horizontal Runners

Left runner					Right runner				
	Lengths of axillaries in mm.		Inequality*	Difference from 100%		Lengths of axillaries in mm.		Inequality*	Difference from 100%
	Orthostichy		Of axillaries at upper or left orthostichy			Orthostichy		Of axillaries at upper or left orthostichy	
No. of node on runner	Upper or left	Lower or right			No. of node on runner	Upper or left	Lower or right		
1	5	6	90·9	−9·1	1	2·8	2·4	107·6	+7·6
2	6	1·1	169	+69	2	0·8	2	57·1	−42·9
3	8	12	80	−20	3	2·8	1·15	141·7	+41·7
4	211	1·3	198·7	+98·7	4	1·2	21	10·8	−89·2
5	233	47	166·4	+66·4	5	24	29	90·5	−9·5
6	237	2	198·3	+98·3	6	3	160	3·6	−96·4
7	63	13	165·7	+65·7	7	67	52	112·6	+12·6
8	38	1·8	190·9	+90·9	8	1·7	31	10·3	−89·7
9	3·2	4	88·8	−11·2	9	2·6	3	92·8	−7·2
10	5·4	1·4	158·8	+58·8	10	0·38	0·9	59·3	−40·7
11	1·15	1·2	97·8	−2·2	11	0·21	0·22	97·6	−2·4
12	0·4	0·2	133·3	+33·3	12	0·06	0·13	63·1	−36·9
13	0·16	0·16	100	0					

* Expressed as percentage difference of these members from the mean value of the particular pair of axillaries concerned.

differences of these members from the 100 per cent. value (i.e. when both of the members of an axillary pair have the same length). Thus where the degree of inequality exceeds 100 per cent. a positive value is obtained and if it is less than 100 per cent. a negative value results.

Fig. 3 shows the resulting positive or negative values of the left or upper members plotted against their nodal number on this pair of runners. From the left runner it can be seen that at the even nodes the left axillaries have always positive values (i.e. are longer than their partners) but that at the odd nodes there is no stable arrangement. Thus the upper axillaries have negative values at nodes 1 and 3, become positive at nodes 5 and 7, again negative at nodes 9 and 11 and finally at node 13 the inequality is zero. The inequality of the members at the odd nodes also shows much less variation from zero than those at the even nodes. Fig. 3 of the right runner shows similar results. However, it will be noted that in this runner the left axillaries at the even nodes are always negative in contrast to the positive values always found in the left runner. There also appears to be an overall tendency in both runners for the axillaries at the younger nodes to exhibit smaller differences from zero than at the older nodes. This appears to be due to an increase in the inequality of a pair of axillary members with time rather than to an actual decrease in the inequality of the axillaries at later formed nodes.

Thus in another pair of runners (older than the pair shown in Fig. 3) nodes 8 to 11 on the left runner and nodes 8 to 10 on the right runner were mature and the axillaries were well developed. At nodes 8 and 10 on the left runner the inequality of the left axillary members (expressed as their differences from the

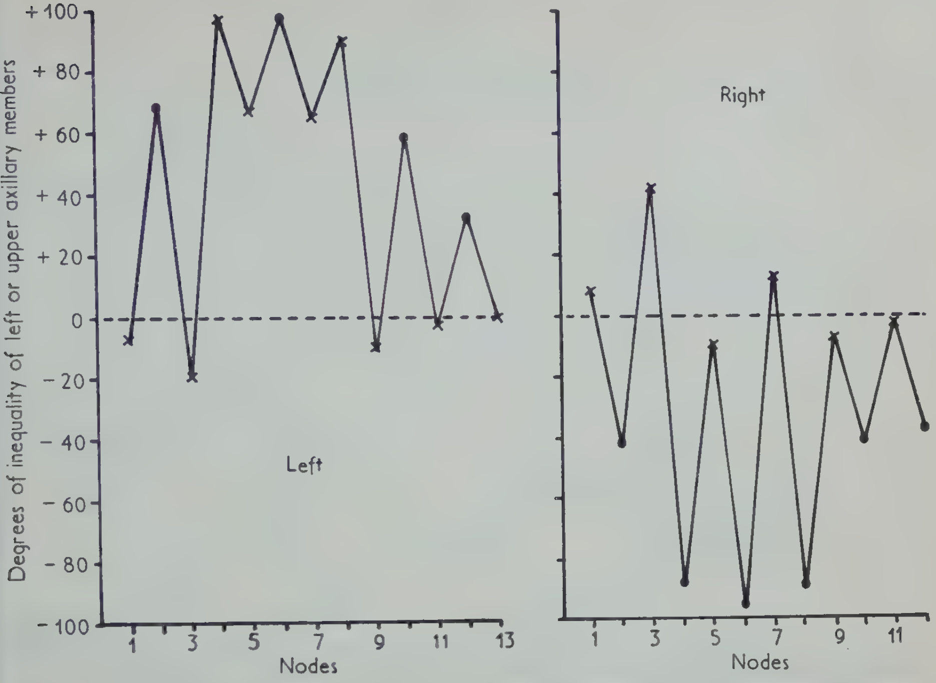

FIG. 3. Degrees of inequality of the left and upper members at the even and odd nodes respectively expressed as their differences from the 100 per cent. value. Odd nodes (**x**) and even nodes (●).

100 per cent. value) are +91·2 per cent. and +93·6 per cent. respectively, and at the corresponding nodes of the right runner are −85 per cent. and −71·5 per cent. At nodes 9 and 11 of the left runner the values for the upper axillaries are +51·5 per cent. and +5·8 per cent. and at node 9 of the right runner the value for the upper member is −51 per cent. If these values are compared to the values of the corresponding nodes of the left and right runners given in Table I it will be seen that there is a general tendency for the degree of inequality of the axillaries to increase with the age of the particular node.

On the twenty-seven complete runners (including the two pairs described in the preliminary observations) a majority of nodes had both axillaries intact. In Table 2 an analysis of the distribution of the longer (or rarely equal sized) axillaries at the upper or lower orthostichies at the odd nodes or the left and right orthostichies at the even nodes is given for these runners. The majority of the nodes were mature. At the even nodes the longer axillaries are invariably distributed on the orthostichies (the left ones of the left runners and the right orthostichies of the right runners) which in the axillary buds from which the runners developed were adjacent to the subtending leaves of these buds. However, at the odd nodes there is no such obvious relationship in the distribution of the longer axillaries. On the left runners the distribution of the longer axillaries at the upper and lower orthostichies occurs more or less

TABLE 2

Analysis of Distribution of Longer Axillary Members at the Nodes of 27 Complete Horizontal Runners

	Pairs of axillaries at even nodes	
	Left runners	Right runners
No. with left axillary longer	55	0
No. with right axillary longer	0	60
No. with equal axillaries	0	0
Total no. of pairs of axillaries	55	60

	Pairs of axillaries at odd nodes	
	Left runners	Right runners
No. with upper axillary longer	28	14
No. with lower axillary longer	31	46
No. with equal axillaries	4	2
Total no. of pairs of axillaries	63	62

randomly but on the right runners there is a preponderence of the longer axillaries on the lower orthostichy. Of the 125 pairs of axillaries at the odd nodes there are only six in which the members of the particular pair are approximately equal in length.

Sometimes the nodes at which the first-order axillaries were present did not lie horizontally but were oblique to the soil surface. Also in the removal of the runners from the soil it occasionally happened that the original orientation of the parent nodes to the soil surface was obscured. Consequently a few of the runners which were termed left were in actual fact right and vice versa. This in no way alters the picture of the distribution of the longer axillaries at the even nodes. As far as the odd nodes are concerned it is not likely to greatly alter the frequencies of the longer axillaries at the upper or lower orthostichies of either left or right runners since any errors would be random and therefore cancel one another out.

Fig. 4 shows the relationship between the lengths of the pairs of the young axillary buds at node 6 (still present within the terminal buds of various runners) and the distance of node 6 from the terminal apices of the various runner buds. The two lots of measurements fall into two distinct groups with no obvious overlap between them. From the slopes of the two best-fitting straight lines fitted to the two groups it is obvious that the rate of increase in length of the longer axillary members with increasing distance from the apex of the terminal bud is greater than for the shorter axillaries.

Table 3 records the lengths of axillaries, and their first order extra-axillary buds, at various nodes visible within a number of cleared terminal buds. The distribution of the longer first-order extra-axillaries of the pair at any node normally follows (except in one example) the distribution of the longer axillary

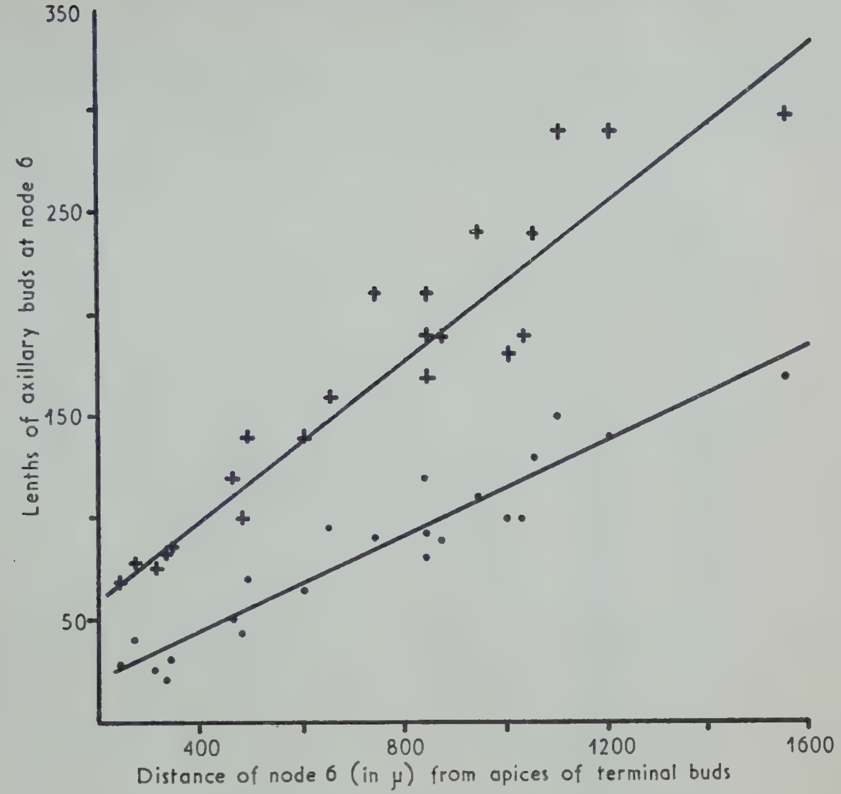

FIG. 4. Lengths of axillary buds (in μ) at node 6 within cleared terminal buds against distance of this node from the apices of the terminal buds. Longer members of each pair (+) and shorter members (•).

TABLE 3

Lengths of Axillary Buds and Their Respective Extra-Axillaries at Nodes Visible in Cleared Terminal Buds*

Length in microns of				Inequality†		Difference from 100%	
Longer axillary	Extra-axillary of longer axillary	Shorter axillary	Extra-axillary of shorter axillary	Longer axillary	Extra-axillary of longer axillary	Longer axillary	Extra-axillary of longer axillary
550	120	380	85	118·2	118·1	+18·2	+18·1
350	80	280	82	111·1	98·4	+11·1	−1·6
650	150	330	100	132·6	120	+32·6	+20
730	190	420	120	126·9	122·5	+26·9	+22·5
900	220	360	140	142·8	122·2	+42·8	+22·2
380	100	210	85	128·8	108·1	+28·8	+8·1
390	97	200	67	132·2	118·1	+32·2	+18·1
470	120	230	57	134·2	135·2	+34·2	+35·2
500	140	470	120	103	107·6	+3	+7·6

* First order.

† Calculated as in Table 1.

buds (Fig. 1 C, D). However, sometimes the degree of inequality of the longer axillary at a node is considerably different from the inequality of the longer extra-axillary bud at that node. Thus there is obviously no rigid parallel between the size of the first order extra-axillaries and that of their parent axillary buds.

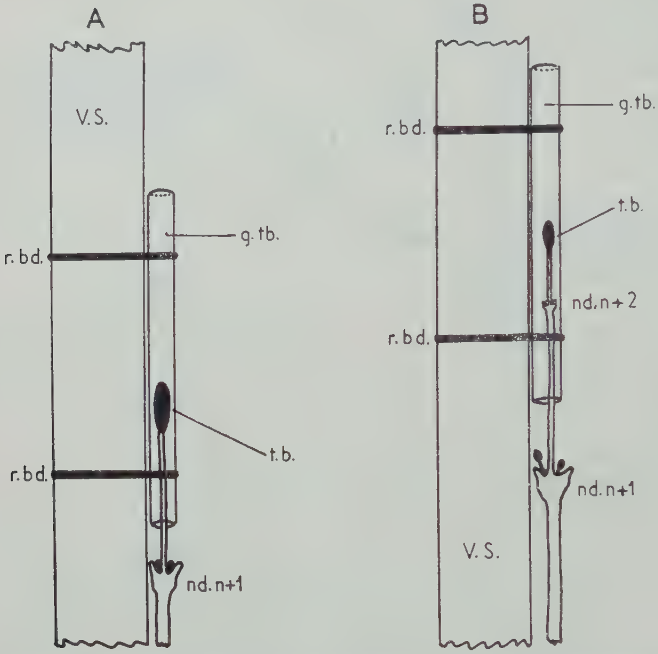

FIG. 5. Diagrams to illustrate the method of growing a runner bud vertically. A, the open-ended glass tube in which the terminal bud grows is attached to a vertical support by a pair of rubber bands. B, the same runner at a later stage with another node now distinct from the terminal bud and the tube raised to allow for the growth of the bud and runner. *g.tb.*, glass tube; *nd.n+1*, node n+1; *nd.n+2*, node n+2; *t.b.*, terminal bud of runner; *v.s.*, vertical support.

THE EFFECT OF GRAVITY ON AXILLARY BUD DEVELOPMENT

Since the vegetative runners generally grow more or less horizontally it seemed possible that the pattern of inequality of axillary members might in some way be due to the effect of gravity. Experiments were therefore conducted in which the terminal buds of runners were grown approximately vertically over a number of plastochrons and the effect of this treatment on axillary production investigated. Fig. 5 illustrates the method employed. A glass tube of about 5 mm. diameter was attached by rubber bands to a vertical support which was firmly embedded in the soil of the pot in which the plant was growing. At the start of a particular experiment a note was made of the number of mature nodes on the runner. In this way it was possible, knowing that the maximum number of pairs of leaf primordia present in a terminal bud is normally five, to establish later in the experiments which of the axillaries on a particular runner were initiated after the beginning of the experiment. The end of the runner and its terminal bud were placed inside the glass tube (Fig. 5A) and as the runner grew the tube was adjusted to accommodate the increasing height of the runner (Fig. 5B). Since the tube was narrow it was also necessary to remove the pairs of young leaves from the terminal bud as they became successively unfolded from it.

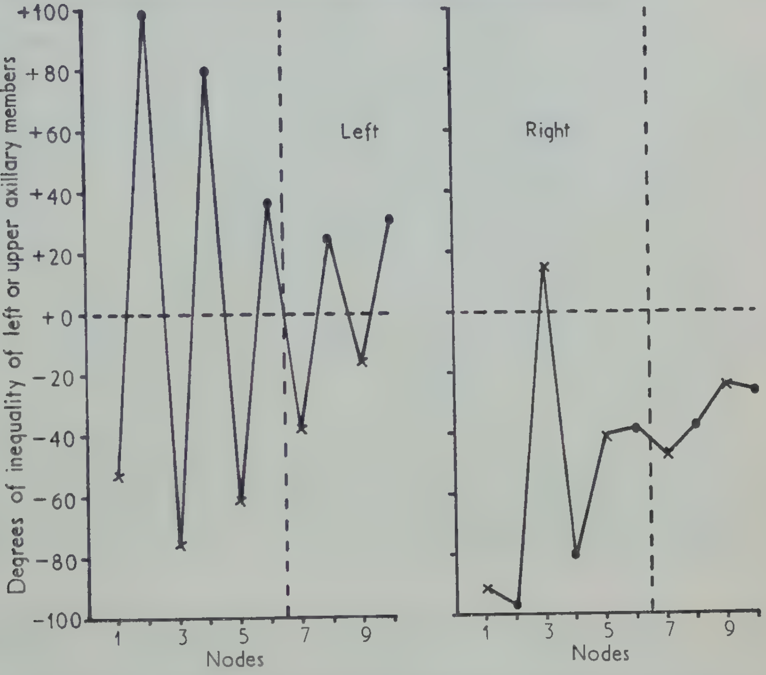

FIG. 6. Degrees of inequality of the left and upper members at the even and odd nodes respectively of a pair of runners expressed as their differences from the 100 per cent. value. The broken vertical lines separate the last developed nodes of the two runners when growing horizontally from the first formed nodes when growing vertically. Odd nodes (**x**) and even nodes (●).

Fig. 6 shows the inequality of the left and upper axillary members of a pair of runners grown in this manner. The vertical broken lines of the figure indicate the oldest node on each runner at which axillary buds could have been initiated before the vertical growth of the terminal buds began. As in the horizontal runners, the left axillaries at the even nodes of the left runner are all longer and on the right runner the left axillaries are all shorter. On these two particular runners the majority of axillaries at the upper orthostichies of the odd nodes were shorter. At the odd nodes there seems to be generally a lower inequality of the axillaries than at the even nodes and there is also an overall tendency for the inequality at both odd and even nodes to decrease at the younger nodes. This former fact is not so obvious on the right runner illustrated in Fig. 6 but nevertheless on various other runners experimented upon there was an obvious difference between the inequality of the members at the odd and even nodes.

A total (including the pair of runners mentioned above) of four pairs of runners, one single runner and six incomplete runners (in which, however, the nodal numbers were known) were subjected to this vertical treatment. It has been seen from the study of the horizontal runners that at the even nodes there is always a high degree of inequality of the axillaries and that the longer members are always on the orthostichy adjacent to the subtending leaf of the axillary bud from which the runner developed. In the nine complete runners this was also the case at all the even nodes, and whilst in the six incomplete runners the left and right orthostichies at the even nodes were not distinguishable, it was found that the longer axillaries on any particular runner were always on the same orthostichy. On these complete and incomplete runners there were a total of thirty-one pairs of axillaries at the even nodes which had been initiated after the vertical treatment began. It is evident that the vertical growth of a runner in no way alters the pattern of development of these axillaries when compared to that found in the horizontally growing runners. With regard to the thirty-six odd nodes formed after the beginning of the experiment it is more difficult to be conclusive since the development of the axillaries at the odd nodes in horizontal runners is much more variable than at the even nodes. However, since in the vertically growing runners the majority of these axillaries at the odd nodes were still unequal in size it appears that gravity does not control the pattern of inequality of the axillaries on the vegetative runners of *Glechoma.*

ANISOPHYLLY IN RELATION TO THE INEQUALITY OF AXILLARY MEMBERS

Superficially anisophylly in *Glechoma* is rarely very obvious, but since it might be expected that whatever factors control the production of unequal axillaries may have a similar effect on their subtending leaves it was decided to investigate this matter more fully.

In Fig. 7 the inequality of the upper and left axillaries (expressed as their individual differences from the 100 per cent. value) and the inequality of their respective subtending leaves, calculated from laminal area, on two pairs of young runners are plotted against nodal number. Normally the leaves show little inequality (except on the right runner of pair B) when compared to the inequality of their respective axillaries. Although in both the runners of pair A and in the left runner of pair B it happens that the patterns of the plotted values of inequality of the leaves compared to the respective axillaries are often similar, it is doubtful whether this has any significance. Thus whilst the inequality of a given axillary member may be positive or negative the inequality of the corresponding subtending leaf often shows an opposite tendency to it. In the right runner of pair B the plotted values of inequality of the leaves are more or less a mirror image of those of their respective axillaries.

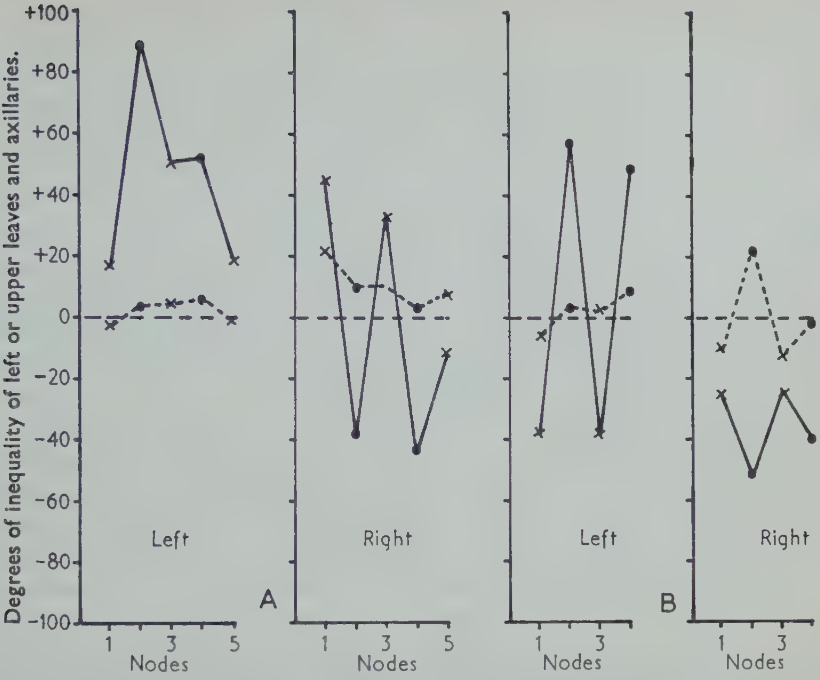

FIG. 7. Degrees of inequality of the left and upper leaves (broken lines) and their respective axillaries (continuous lines) at the even and odd nodes of two pairs of runners expressed as their respective differences from the 100 per cent. value. Odd nodes (**x**) and even nodes (·)

In general therefore, the inequality of the axillaries is much greater than those of their subtending leaves and there is no constant relationship between the inequality of the two.

DISCUSSION

Goebel (1900, p. 111) mentioned that in *Elatostemma sessile* and *Centradenia inaequalateralis*, which are both plants with a decussate phyllotaxy, one leaf of a pair is always much larger than the other. An axillary shoot develops only in the axil of the larger leaf. Champagnat (1948, pp. 169–70) described other examples of this inequality. Thus in oblique shoots of *Aesculus* the lower sides have larger leaves than the upper sides and in any pair of axillary buds the larger occurs in the axil of the larger of the pair of leaves. He also stated that in *Fraxinus*, *Acer*, and *Sambucus* the same phenomenon occurs. By contrast in *Pilea muscosa* Lindl. the larger axillaries are found in the axils of the smaller leaves. In *Cuphea* although the inequality of the axillary pairs is obvious from early in the development of the plant, a parallel inequality of the leaves subtending these pairs of axillaries only develops later at the younger nodes.

In *Dianthus caryophyllus*, variety Maytime, the number of pairs of axillary buds at each node from nodes 1 to 6, on twenty axillary branches, was recorded by Shushan and Johnson (1955). At nodes 6 and above only a single bud was found per node but at older nodes pairs of axillaries (instead of single buds) were more common at the odd than the even nodes. At nodes where a pair of buds were present usually one of the buds was larger than the other. If the presence of only one bud per node is considered to be the extreme form of inequality then it follows that at the even nodes below node 6 there is on the whole a greater inequality of the axillary buds than at the odd nodes. Since no mention was made as to the position of the larger bud (or if only one was present, the single bud) at node 2 no direct comparison can be made with the situation in *Glechoma*. However, it was stated that the larger (or single buds) are generally arranged in a spiral and that in any particular orthostichy larger buds only occur at intervals of every four nodes. Thus the larger buds are not confined to one straight orthostichy as they are at the even nodes in *Glechoma*.

In *Acer pseudoplatanus* anisophylly is already evident in the leaf primordia at the even numbered nodes of the young axillary buds on the main axis of seedling plants (White, 1955 and 1957). It is also evident in the mature leaves of the first-year lateral shoots which develop from these buds. This anisophylly White termed primary and considered it to be due to the spatial conditions within the leaf axil where the axillary bud develops. He contrasted this primary anisophylly with the secondary anisophylly which is also to be found in *Acer*, but to a lesser and variable extent, in the shoots which develop in the next season from the isophyllous winter buds. These buds are formed at the tips of the first-year lateral shoots at the end of the season's growth. White agreed with Sinnott and Durham (1923) that this secondary anisophylly is probably due to gravity.

The first-formed pair of leaves of an axillary bud in *Acer* (as in *Glechoma*) are orientated at right angles to the plane of the parent leaf axil. Both these and other pairs of leaves subsequently formed in the same two orthostichies show little anisophylly in contrast to the considerable degree of anisophylly of the leaf pairs at node 2 and later formed even numbered nodes. At these even nodes the larger leaves invariably occur on the orthostichy of the axillary axis adjacent to the subtending leaf. It is interesting to note that Goebel (1900, p. 112) described a similar case in *Goldfussia glomerata*. In this decussate plant the leaf pair at node 2 of an axillary bud are unequal in size and the larger is also adjacent to the subtending leaf. In *Acer* all of the leaves unfolded in the extension growth of the first-year lateral shoot are initiated whilst the axillary bud still lies within the leaf axil. In the seedling plant the axillary bud lies more or less vertically, and at least in the early stages of development the axillary bud lies hidden within the terminal bud. White therefore considered that neither gravity nor light could be the cause of this primary anisophylly. Similarly he found no evidence that unequal nutrition of the two sides of the axillary apex occurs to account for this phenomenon.

White concluded that primary anisophylly is in fact caused by the unequal pressure on the axillary apex due to the lack of space in the leaf axil where the axillary bud develops. Thus those pairs of leaves to develop on the axillary apex in the plane of the parent leaf axil (i.e. those at nodes 2, 4, &c.) are always markedly anisophyllous. The leaves at these nodes formed on the side of the axillary apex next to the parent shoot axis are smaller since the pressure on this side of the apex is said to be greater than on the other side. Presumably on this hypothesis the low degree of anisophylly of the pairs of leaves at the odd nodes which becomes evident in the extension growth of the axillary shoot (but which is not evident in the axillary bud) is due to random variations in the growth-rates of these leaves.

In *Glechoma* there is no evidence that the generally greater inequality of the axillaries at the even compared to the odd nodes of a runner, results from an unequal pressure on the apex of the axillary bud from which the runner develops. Even if such an unequal pressure were to exist, it would seem unlikely to affect the axillaries but not their subtending leaves. It would be interesting to know whether in this species of *Acer* the marked anisophylly of the leaves at the even nodes which White described is accompanied by a parallel inequality of the axillaries subtended by them. Under greenhouse conditions the vegetative runners of *Glechoma* are persistently monopodial and grow throughout the year. There is no evidence that the inequality of the axillary members at the mature nodes, which have been formed by the terminal bud after the runner has developed from the axillary bud, is in any way different to that of earlier formed axillaries. White considered that the isophyllous condition of the winter bud formed at the end of the first year's growth of the axillary shoot confirmed his theory as to the importance of spatial conditions in the parent axil in determining primary anisophylly. The evidence of the persistent inequality of the axillaries at the younger nodes indicates that this is not true in *Glechoma*. White further mentioned that the first-year lateral shoot formed by the extension growth of an axillary bud always exhibits anisophylly. It is not altogether clear whether this is generally true, or whether his statement was meant to apply to the lateral shoots arising as axillary members from the main axis of the seedling plant. If this latter is the case it would be interesting to know whether primary anisophylly is also to be found in first-year lateral shoots arising from the branches of an old tree.

The analysis given in this paper of the occurrence of inequality between the two individuals of a pair of axillary members in *Glechoma* indicates that it is not in any way controlled by gravity. Further the varying, but generally small and apparently random, degree of anisophylly of the subtending leaves do not seem to be connected with the inequality of their respective axillary members. As in the phenomenon of anisophylly in *Acer*, light cannot be considered as a possible cause of the inequality of axillary members in *Glechoma* since the formation of these unequal axillaries has already occurred whilst within the parent terminal bud.

The possibility exists, however, that this inequality might be produced by the unequal distribution of some metabolite at the apex of the axillary bud, or the runner which develops from it. Williams (1960) has shown that the application of the anti-auxin TIBA (2:3:5-tri-iodobenzoic-acid) had a striking effect in reducing the growth curvature of the radicles of *Striga asiatica*.

This curvature is evident in germinating seedlings of this plant and Williams further showed that it was not altered by rotation on a horizontal klinostat. As a result of these experiments it was suggested that some relatively immobile growth hormone (probably with an inhibitory effect upon cell extension in the root) is the cause of this curvature. It is possible to imagine a similar kind of hormone (which is not in anyway affected by gravity) which, if present, might cause the inequality of development in the axillaries of *Glechoma*. Thus, if for some reason a greater concentration of this hormone were present on the side of the terminal apex of the runner whose orthostichy is adjacent to the side of the parent node of this runner, it might inhibit axillary development. Hence at the even nodes the orthostichy adjacent to the subtending leaf of the axillary runner would always have longer buds than on the opposite orthostichy. At the odd nodes the lower degree of inequality of the axillaries and the less stable arrangement of the longer axillaries might also be explained. Thus on this hypothesis it is likely that on the other two sides of the runner apex the differences of concentration of the growth hormone would not be so great. Therefore the inequality of the axillary members would be smaller and there would be more chance of a variation occurring in the position of the longer axillary.

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